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Systematics of the *Viola nuttallii* Complex

by

D. M. Fabijan



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Plant Taxonomy

Department of Botany

EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Systematics of the *Viola nuttallii* complex submitted by D. M. Fabian in partial fulfilment of the requirements for the degree of Master of Science.

Date ..... *December 12, 1983*



## Abstract

Morphological, cytological and phytochemical data were incorporated into numerical and statistical analyses resulting in a revision of *Viola* subsection Nuttallianae. Five species, with three subspecies, were recognized. A basic chromosome number of  $x = 6$  was confirmed in this polyploid complex which includes: *V. vallicola*,  $2N = 12$ ; *V. tomentosa*,  $2N = 12$ ; *V. nuttallii*,  $2N = 24$ ; *V. praemorsa*,  $2N = 36$  and  $48$ ; and, *V. bakeri*,  $2N = 48$ . A total of twenty-nine flavonoid compounds were found in leaf tissue extracts from these eight taxa. Identified flavones and flavonols included the arabinose, galactose, glucose, rhamnose, and rutin glycosides of apigenin, kaempferol, luteolin and quercetin. The number of different flavonoid compounds per taxon increased with ploidy level within this complex.

*V. vallicola* subspecies *vallicola* possessed primarily apigenin glycosides and occurred in Alberta and Saskatchewan. Subspecies *major*, on the other hand, was found to possess predominantly kaempferol glycosides and occurred only in the interior of British Columbia. A number of specimens of *V. vallicola* from the Great Basin regions of Idaho and Nevada displayed intermediate flavonoid profiles. The derivation of the subspecies is hypothesized to be a result of isolation on either side of the continental divide during post-glacial northward migration.

*V. nuttallii* possessed apigenin, kaempferol and quercetin flavonoids suggesting that it is an allotetraploid of *V. vallicola* subspecies *vallicola* and *V. tomentosa* which also possessed apigenin, kaempferol and quercetin derivatives. *V. tomentosa* has since become restricted in range to the Sierra Nevada mountains and *V. nuttallii* is found only on the east side of the Rocky Mountains. *V. vallicola* has the widest distribution of any of the Nuttallianae, ranging from southern British Columbia to Manitoba and south to Arkansas and Nevada.

*V. bakeri* and *V. praemorsa* possessed the most diverse flavonoid profiles, up to twenty per taxon, including luteolin aglycone and glycosidic derivatives. Presence or absence of different flavonoid aglycones appeared to be random in the specimens in these taxa. The three subspecies of *V. praemorsa*: *praemorsa*, *linguaefolia*, and *f/avovirens*, were recognized by their morphological characteristics, though they appear to represent extremes in a continuous range of variation. The existence of hybrids



supports the hypothesis that other previously recognized subspecific taxa, subspecies *arida* and *oregona*, are the result of hybrid crosses of *V. praemorsa* and *V. bakeri* or with members of closely related subsections.



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## I. INTRODUCTION

### A. General Introduction

The *Viola nuttallii* complex of the family Violaceae has been identified as a polyploid series; the taxa in this group contain meiotic chromosome complements of 6, 12, 18, and 24. Due to their morphological similarities, they have been difficult to distinguish, resulting in the description of twenty different taxa since *Viola nuttallii* was described in 1814. The *Viola nuttallii* complex thus provided a challenging taxonomic problem.

### 1. The Family Violaceae

The family Violaceae consists of 16 genera and approximately 800 species of worldwide distribution (Cronquist, 1981). Fourteen of the sixteen genera in the family are restricted to the southern hemisphere and particularly to South America.

Melchior (1925) divided the family into two subfamilies based primarily on floral characters. Leonideae have terminally opening stamens and nut-like fruit, while the Violoideae have introse stamens and capsular fruit. The subfamily Violoideae was further divided into two tribes; the Violeae Gingins was distinguished from the Rinoreae Reiche and Taubert by the possession of zygomorphic flowers in which the lower petal was saccate or spurred. The tribe Violeae was further divided into the subtribes Hybanthinae with two genera, and the Violinae with five genera including *Viola* L. The Violinae are distinguished by a relatively long spurred anterior petal and distinct stamens with appendages extending into the spur.

### 2. The Genus *Viola* L.

The nearly 400 species of the family Violaceae belong to the genus *Viola*, which is composed primarily of annual and perennial herbs, although shrubby and arborescent species are found in South America (Camp, 1947). Whereas actinomorphic flowers predominate in other genera of the Violaceae, those of the genus *Viola* L. are strongly irregular (Clausen, 1929; Cronquist, 1981).



Camp (1947) proposed Central and South America to be the area of origin of the Violaceae and of the genus *Viola*. He based this hypothesis on the southern distributions of many of the genera in the family, particularly the *Rinorea* which were considered primitive within the family (Cronquist, 1981).

The only genus within the family which is best developed in north temperate regions and in tropical mountains is *Viola* (Figure 1). Its South American members have been shown to possess the greatest number of primitive character states that are most closely related to other members of the Violaceae (Clausen, 1929). Camp (1947) contended that *Viola* was "evolutionarily preconditioned" at high elevations in the Andes for its dispersal via Mexico and North America, into the northern hemisphere.

The approximately 400 species in the genus *Viola* were divided into 14 sections by Becker (1925). Six sections occur in the northern hemisphere, and of these, three are found in North America (Valentine, 1962). The sections Chamaemelanium Ging. (Figure 1) and Viola are distributed in both Asia and North America, while Melanium Ging. is primarily European with one species occurring in North America.

### 3. The section Chamaemelium

The section Chamaemelium contains the greatest number of western North American *Viola* species (Baker, 1949c). Violets of the Chamaemelium section are distinguished from those of other northern sections by their small stipules, yellow or yellowish flowers with capitate styles, and the presence of cleistogamous flowers on aerial stems (Becker, 1925; Baker, 1935 and 1949c).

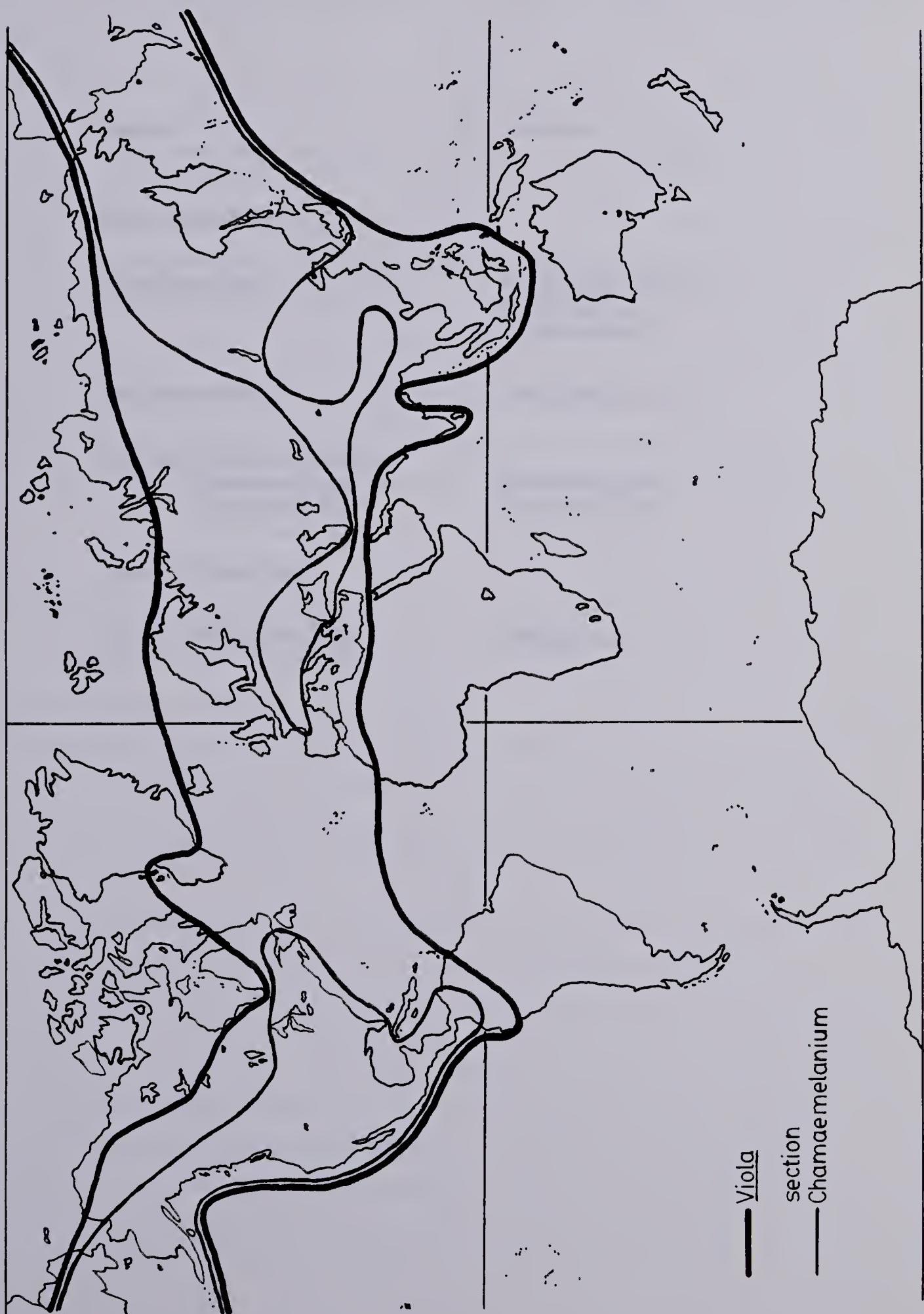
Two classifications of the subsections in the Chamaemelium are shown in Table 1.

Clausen (1964) recognized three Chamaemelium subsections: the Pedunculatae, the Purpureae, and the Nuttallianae whereas Becker (1925) had grouped all of the species into one subsection, Nuttallianae. The Nuttallianae and Purpureae, which were both composed of large species complexes, had previously been grouped into one subsection, Nuttallianae (Baker, 1949c), which was otherwise identical to Clausen (1964).

The Nuttallianae are distinguished by the presence of glabrous capsules as compared to the appressed, puberulent capsules of the Purpureae; by generally lacking



Figure 1. The worldwide distribution of *Viola* and the section Chamaemelanium Ging. (Clausen, 1929; Camp, 1947). (adapted from)





**Table 1.** The subsections of the *Chamaemelanium* Ging. (Becker, 1925; Clausen, 1964).

Becker	Clausen
Barroetanae Bckr.	
Nuttallianae Bckr.	Nuttallianae (Bckr.) Cl. Purpureae Cl. Pedunculatae Cl.
Chrysanthaes Bckr.	Chrysanthaes Bckr.
Erectae - Monophyllos Bckr. - Nudicaulis Bckr. - Canadensis Bckr.	Nudicaulis Bckr. Canadensis Bckr.
Flagelliformes Bckr.	
Section Dischidium Ging.	Biflorae Cl.



elongated aerial stems; and, by size and shape differences in the basal leaves (Baker, 1957; Clausen, 1951; 1964). Further, in contrast to the Purpureae ( $n = 6$ ), the Nuttallianae constituted a polyploid species complex with chromosome numbers of  $n = 6$ , 12, 18 and 24.

Members of the Chamaemelanium section found in western North America and Mexico may represent the prototypes of the temperate *Viola*, constituting a link between South American tropical members and the other northern temperate violets (Clausen, 1929; 1964). The species of the Chamaemelanium were judged to be the most primitive of the *Viola* on the basis of their low chromosome number of  $n = 6$ , and the presence of variations in style shape, stigma form, and leaf form found in other sections (Clausen, 1929). Cytological and morphological data coupled with geographic distribution led to a phylogenetic proposal for the northern violets by Clausen (1929), in which the Chamaemelanium section was thought to be the central group from amongst whose members the ancestors of the other northern subsections and sections might have been found.

## B. The *Viola nuttallii* Pursh Complex

### 1. Historical Introduction

*Viola nuttallii* Pursh was first collected by Thomas Nuttall in 1811 while he was on the Astor Expedition on the Missouri River. Pursh's (1814) publication described a pubescent unbranched plant having ovate-oblong, nearly entire leaves with long gradually tapering petioles.

In 1829, Lindley published the description of a new species by D. Douglas, *Viola praemorsa* Dougl. ex Lindl. It was named for its truncate rootstock, and was distinguished from *V. nuttallii* by possessing denticulate, ovate-oblong leaves covered by hirsute hairs. *V. praemorsa* was found to be abundant on the dry plains of the Columbia River and especially along the Pacific Coast. It was only one year later that Hooker (1930) included in this species a rare form from the Columbia River area, *V. nuttallii* Pursh variety *major* Hook. with wide leaves and large flowers and growing in the shade of pines. He stated that this taxon was related to the collections of Nuttall from the



Missouri River. Hooker also synonymized *V. glareosa* Dougl. with variety *major*, though it was never published.

*The Flora of North America* by Torrey and Gray (1843) included yet another species description by Nuttall of a plant which he collected in 1834 while accompanying the Wyeth Expedition. *V. linguaefolia* Nutt. ex T. & G. possessed pubescent, oblong-lanciolate and somewhat serrate leaves, being intermediate between those of *V. nuttalii* and *V. praemorsa*.

Based on collections of Heller in Idaho in 1896, Pollard (1897) described *V. flavovirens* Poll. as a tall yellowish-green plant covered with fine hirsute pubescence.

In 1898, *V. bakeri* Greene was described as a pubescent form with entire, ciliate margined leaves intermediate between *V. nuttalii* and *V. praemorsa* from California.

*V. vallicola* A. Nels. was not described until Nelson (1899) distinguished it from *V. praemorsa* by its finely puberulent or glabrous character and peduncles which did not elevate the flowers above the height of the leaf blades. In 1900 Nelson described another related species from subalpine habitats in Wyoming and Idaho. This species, *V. erectifolia* A. Nels., was distiguished from *V. nuttalii* by its erect leaves being somewhat wider and with a single vertical taproot. Otherwise, *V. erectifolia* appeared to be very similar to the *V. nuttalii* represented by the drawing in Hooker (1830).

Other species continued to be described as related to *V. nuttalii*, for example: *V. gomphopetala* Greene and *V. physalodes* Greene (Greene, 1901), *V. subsagittifolia* Suks. and *V. xyloorrhiza* Suks. (Suksdorf, 1927), and *V. russelii* Boivin (Boivin, 1951). New combinations also began to appear for previously described taxa: *V. nuttalii praemorsa* (Piper, 1906), *V. nuttalii linguaefolia* Piper (Piper and Beattie, 1914), *V. nuttalii* var. *vallicola* (St. John, 1937), *V. praemorsa* var. *linguaefolia* (Peck, 1941), to mention but a few.

## 2. Cytological Investigations

With the advent of cytological methods, new emphasis was placed on chromosome numbers in taxonomic work. Amongst the first plants investigated were members of the genus *Viola* (Miyaji, 1913, in German translation, 1930). Miyaji, in a study of Japanese violets, found meiotic chromosome numbers of 6, 12, 18, 24 and 36



present in the genus. The cytology of North American violets was first investigated in conjunction with the hybridization studies occurring in the early years of this century (Brainerd, 1904; Gershoy, 1928; 1934).

It was also at this time that Milo Baker, in collaboration with Jens Clausen, began a revision of western North American violets using cytological techniques. Their determinations, which were included in numerous publications between 1935 and 1960, were subsequently summarized with documentation by Clausen (1964). As a result, a number of new combinations were made and new taxa described. *V. tomentosa* Baker (1949a),  $n = 6$ , a rare endemic of the Sierra Nevada was similar to *V. bakeri*,  $n = 24$ , and *V. nuttallii*,  $n = 12$ , but with densely tomentose leaves and capsules. *V. bakeri* subspecies *grandis* Baker (1949a), was described as a larger leaved form of *V. bakeri*. *V. praemorsa* subsp. *linguaefolia* (Nutt.) Baker and Clausen (Baker, 1957),  $n = 18$ , and *V. praemorsa* subsp. *major* (Hook.) Baker and Clausen (Baker, 1957),  $n = 24$ , represent two new combinations. *V. praemorsa* subsp. *arida* Baker (1957),  $n = 24$ , was very similar to subsp. *praemorsa* except for its short and sparse pubescence on leaves and capsules. *V. praemorsa* subsp. *oregona* Baker (1957),  $n = 24$ , was found in a few isolated areas in southern Oregon and northern California; it differed from *arida* by having spreading instead of erect foliage, and minutely puberulent capsules.

To this point each taxon was represented by only one or very few chromosome counts. Davidse (1976), in the most recent work on this group, undertook an extensive cytological investigation of the members of the Chamaemelium section, including some Nuttallianae, in the Intermountain Region between the coastal Sierra Nevada mountains and the Rocky mountains. He confirmed all of the above reports except for *linguaefolia* for which he acquired nine counts of  $n = 24$  (Table 2).

### 3. Classification of the Nuttallianae

As a basis for discussion, and by way of a summary of the most extensive work done to date, the classification of Clausen (1964) will be reviewed. This classification included *V. tomentosa* Baker, *V. bakeri* Greene, *V. nuttallii* Pursh, *V. vallicola* A. Nels., *V. linguaefolia* Nutt. ex T. & G., and *V. praemorsa* Dougl. ex Lindl. with three subspecies: *praemorsa*, *major* (Hook.) Baker and Clausen, and *oregona* Baker and Clausen.



**Table 2.** Cytology of the Nuttallianae and reported hybrids.



The classification of the Nuttallianae by Clausen (1964) has not, however, been accepted universally. Several authors have chosen to designate all taxa uniformly as varieties (Hitchcock and Cronquist, 1961; Scoggan, 1978) or subspecies (Peck, 1961) of *V. nuttallii* due to the lack of sufficient cytological data and the absence of reliable morphological or geographical differences in the cases of some taxa.

In his synopsis of the western North American violets, Clausen (1964) concluded that the 33 taxa within the Chamaemelium section belonged to at least seven polyploid lines which represented distinct evolutionary sequences. The subsection Nuttallianae comprised two of these polyploid lines, based on two geographically separated diploid species.

The first polyploid line was based on *V. tomentosa*,  $n = 6$ , a rare endemic of the Sierra Nevada (Baker, 1949a). This line was thought to be more closely related to the subsection Purpureae (*V. purpurea*, *V. quercetorum*, and *V. utahensis*) than to other members of the Nuttallianae. *V. tomentosa* was presumed most closely related to *V. bakeri*,  $n = 24$ , based on morphological characters, although no hexaploid species was known with which it could have hybridized to produce *V. bakeri*.

The second series included *V. vallicola*, *V. nuttallii*, *V. praemorsa*, and *V. linguaefolia*. Clausen suggested that this line arose from *V. vallicola*.

*V. nuttallii*,  $n = 12$ , is a prairie species that remains east of the continental divide in a range extending from the Canadian prairie provinces as far south as Arizona and as far east as Nebraska. It is distinguished by its lanceolate leaves which are attenuated to a long petiole and minutely puberulent throughout, and by arising from a deep-seated rootstock with renewal buds below the soil surface.

*V. vallicola*,  $n = 6$ , has the widest distribution of any taxon in the complex. It is sympatric with *V. nuttallii*, but also ranges into central British Columbia, Washington and Oregon. It has a truncated, vertical rootstock and ovate or oblong leaves which tend to have truncate or subcordate bases (Baker, 1957).

Baker (1957) suggested that *V. nuttallii* was an autotetraploid of *V. vallicola*, although Clausen (1964) questioned this conclusion based on their morphological distinctiveness. He suggested that an undiscovered diploid *V. nuttallii* might exist from which the known tetraploid arose via autoploidy. No such diploid has been



discovered and its existence was considered to be unlikely by Davidse (1976).

*V. praemorsa* subspecies *praemorsa*,  $n = 18$ , occurs in the humid regions of the Pacific coast from California to southwestern British Columbia. It was named for the *praemorse* habit of its roots and is distinguished by its dense, hirsute pubescence throughout, its oblong-ovate leaves with truncate bases, and flowers on peduncles which surpass the leaves in height.

*V. /inguæfolia* is a montane to subalpine violet of the Great Basin Ranges and Rocky mountains, extending northward into Alberta, geographically separated from subspecies *praemorsa*. It possesses ovate leaves with cuneate bases that are between *V. vallicola* and *V. nuttallii* in form. This suggested to Clausen (1964) that */inguæfolia* may be a natural amphiploid of these two species.

*V. praemorsa* subspecies *major*,  $n = 24$ , was morphologically very difficult to distinguish from *V. /inguæfolia*,  $n = 18$ , the only reliable means being cytological. Clausen found it to differ from subspecies *praemorsa* by its erect ovate or oblong leaves which equal or surpass the flowers in height. Subspecies *major* is found from Washington to central California through the Cascade and Sierra Nevada mountains.

Baker (1949b) reported *V. /inguæfolia* to be a hexaploid,  $n = 18$ , like *V. praemorsa* subspecies *praemorsa*. He postulated that *praemorsa* probably differentiated from */inguæfolia* as a result of westward dispersal through the Columbia Gap to the coast (Baker, 1957). Clausen (1964), on the other hand, suggested that the rise of the Cascade mountains lead to the present geographic separation of these two hexaploids which were once connected across the low plains.

Davidse (1976) could not corroborate early reports of  $n = 18$  for */inguæfolia* from nine populations in Utah, Nevada, and Idaho. Each population had a count of  $n = 24$  with regular meiosis and high pollen fertility. As no reliable morphological or geographical differences could be found, he considered *V. /inguæfolia* to be synonymous with *V. praemorsa* subspecies *major*. Davidse further suggested that the previous count of  $n = 18$  from Utah may have been based on a hybrid between *major* and *V. utahensis* Baker and Clausen (subsection *Purpureae*), similar to the one reported by him (Table 2).



*V. praemorsa* subspecies *oregona* has a chromosome complement of  $n = 24$  and reportedly occurs in limited areas of southern Oregon and northern California. Leaves of this taxon are grayish in colour due to a continuous coat of pubescence and similar in form to the ovate- or oblong-lanceolate leaves of *V. bakeri*. Baker (1957) referred to *oregona* as a subspecies in the making and that as such, it was probably the youngest taxon of the *V. praemorsa*.

### C. Objectives

The major objective of this study was to attempt to construct a revised classification of the *Viola nuttallii* complex. Related to this was the need to investigate the relationship between *V. vallicola* and *V. nuttallii*, and, between these taxa and the remainder of the group.

In order to achieve this, a cytological survey of the members of this complex was undertaken, particularly of those members which occur in Canada. Detailed morphological investigations of the characteristics of this group were also carried out in order to establish a usable classification based on readily observable features which are statistically significant in distinguishing between taxa. Flavonoids present in the leaf tissue of the members of the *V. nuttallii* complex were also examined in order to supplement and corroborate the morphological data.

Numerical techniques provide a powerful tool by which to analyze complex phenetic relationships among groups of organisms (Sokal and Sneath, 1978). Although the resultant classification would be based on data not ordinarily used in routine field identification, it must be correlated with readily observable morphological characters to be of general use. Numerical techniques have the ability not only to supply a classification but also to statistically determine which characters are "good" in the taxonomic sense. These objectives were based on the need to use a holistic approach, that is, to investigate as many aspects of the problem as was practical.

The operational species concept applied in this study was a practical one, following that of Cronquist (1978): "species are the smallest groups that are consistently and persistently distinct, and distinguishable by ordinary means." This concept incorporates the working assumptions of many plant taxonomists. The smallest groups



are those which cannot be further subdivided and still conform to the definition. Consistent features exhibited by an individual must fit into the range of variation within the group, and, group variation must have a discontinuity with the variation exhibited by other groups. These group variations must be reasonably persistent between generations and must be distinguishable by means ordinarily used by a botanist, that is, using a hand lens. Reproductive isolation is implied by the definition, since phenetic discontinuity cannot be maintained in the absence of some barrier to interbreeding.

For the remainder of this discussion, the taxa in question will be referred to by their specific epithets only, to avoid any confusion as to their taxonomic status.



## II. Materials and Methods

### A. Herbarium Loans

Specimens from the following herbaria were examined: Arnold Arboretum of Harvard University (A); University of Alberta (ALTA); British Museum, London, England (BM); National Museums of Canada (CAN); Dudley Herbarium (DS) and California Academy of Sciences (CAS), Stanford University; Agriculture Canada (DAO); Gray Herbarium of Harvard University (G); University of Idaho (ID); Jepson Herbarium, University of California (JEPS); KEW Botanical Gardens, England (KEW); Missouri Botanical Garden (MO); University of Pennsylvania (PENN); Academy of Natural Sciences of Philadelphia (PH); Rocky Mountain Herbarium, University of Wyoming (RM); W.P. Fraser Herbarium, University of Saskatchewan (SASK); University of Calgary (UAC); University of British Columbia (UBC); University of California (UC); Smithsonian Institute, U.S. National Herbarium (US); University of Regina (USAS); University of Victoria (UVIC); British Columbia Provincial Museum (V); Willamette Herbarium (WILLU) of Oregon State University (OSU); and, Marion Ownbey Herbarium, Washington State University (WS).

Abbreviations follow those used by Holmgren and Keuben (1974).

### B. Collections

#### 1. Field Studies

Field collections were made throughout as much of the reported range of the *V. nuttallii* complex as was possible during the spring and summer of 1981 and 1982. This included the Canadian range, extending from Vancouver Island through southern British Columbia, Alberta, and Saskatchewan; and selected sites in the United States Pacific Northwest including Washington, Oregon, California, Idaho, and Montana. At each collection site, representative members of the population were sampled and pressed for voucher specimens. Others were collected in bulk and air dried for chemical analysis. Pollen and flower buds were sampled and preserved. At least two live plants were potted for transplantation. All materials were subsequently transported to the University of Alberta for cultivation in the greenhouses, deposition of voucher specimens, and / or



detailed chemical and morphological analysis.

## 2. Greenhouse Cultivation of Live Material

Plants in the greenhouse were maintained under a 16 hour photoperiod throughout the year, supplemented with high intensity discard (HID) lamps which provided a minimum intensity of  $80 \text{ uE.m}^{-2}\text{s}^{-2}$ . Temperature was maintained at  $18^{\circ}\text{C}$  during the day and  $15^{\circ}\text{C}$  during the night. Relative humidity was held constant at 60%.

Some plants were overwintered naturally outside while others were induced into dormancy and stored in a freezer (McPherson, 1972). The dormancy induction schedule covered nine days during which the plants were brought from  $18^{\circ}\text{C}$  to  $-2^{\circ}\text{C}$ : three days at  $12^{\circ}\text{C}$ , two days at  $8^{\circ}\text{C}$ , three days at  $2^{\circ}\text{C}$  to  $4^{\circ}\text{C}$  in darkness, then watered well, placed in sealed plastic bags and stored at  $-1^{\circ}\text{C}$  to  $-4^{\circ}\text{C}$  in the dark. After five weeks, the plants were removed from the freezer and returned directly to the greenhouse growing regime. The survival of plants subjected to both natural and artificial overwintering conditions was about 35% for *V. vallidicola* and *V. nuttallii*. *V. praemorsa* specimens, which grew well under greenhouse conditions, died back to the roots and regenerated repeatedly throughout the year, and were not subjected to overwintering procedures. *V. nuttallii* and *V. vallidicola* also underwent a period of dormancy naturally in the greenhouse but did not regenerate until the following March.

The overall survival of collected specimens following transplantation and cultivation was about 80%. Difficulty of cultivation of these taxa over long periods was reported by Baker (Clausen, 1964).

Seeds were collected from cultivated plants by bagging the expanding capsules in muslin, allowing for retention of the seeds after the dehiscence of the capsule.

## 3. Cytological Studies

Mitotic chromosome counts were made on root tip squashes prepared from actively growing roots using a modification of the method of Tijo and Levan (1950). Root tips were treated in a 0.002M solution a 8-hydroxyquinoline for 2 to 2.5 hours at 14 to  $17^{\circ}\text{C}$ . Tips were washed in distilled water for 10 minutes, transferred to a watch glass containing aceto-orcein and 1N hydrochloric acid (9:1 by volume) for 30 minutes.



The solution was warmed over an alcohol flame briefly 3 to 5 times in 30 minutes. Tips were then placed on a microscope slide in 45% acetic acid. All but the apical 1mm of the root tip was removed, a cover slip applied, and the preparation was squashed by tapping the coverslip several times. Semi-permanent slides were made by ringing the coverslip with a melted mixture of gum mastic and parafin wax (2:1 by volume). Chromosomes were examined under oil emersion with a green filter using an Olympus BHA,PM-10-M photomicrographic system and photographed with an Olympus C-35 camera.

Cytological examination of pollen mother cells was carried out using the iron-aceto-carmine method (Clausen, 1929; Radford et al., 1974; Sharman and Sharma, 1980). Flower buds were collected in the field and fixed in Carnoy's solution (chloroform:ethanol:acetic acid, 4:3:1 by volume) then transferred to 70% ethanol and stored at -4° C until slide preparation and examination.

#### 4. Pollen Viability Tests

Pollen was collected in the field by dissecting anthers from chasmogamous flowers and placing them directly into small vials containing lacto-phenol cotton blue stain (Radford et al, 1974). Vials were stored under refrigeration for up to four months prior to examination. Pollen stored in this manner showed no significant difference in viability or size from that stained and examined fresh. Pollen was examined at 400X magnification; the diameter was measured using a calibrated ocular micrometer.

Viability was estimated as the percentage of pollen grains that were stained dark blue in the 100 grains examined. The diameter of 100 viable pollen grains was measured in up to five pollen samples collected at random from each flowering population. Significance of means was tested using the t-test (Campbell, 1974).

#### 5. Morphological Measurements

Characters were selected on the basis of previous authors' treatments of the *nuttallii* complex (Baker, 1935, 1949b; Clausen, 1964); those used in other descriptions of the taxa; and, through the examination of a wide range of living and herbarium material. As a result, 19 morphological characters were selected for scoring the specimens.

Each quantitative character was measured a minimum of ten times. Characters were



scored at the same relative position and developmental stage on each plant.

Measurements of leaf characters (length, width, and basal angle), flower size (length of lower petal including the spur in chasmogamous flowers), length of cleistogamous sepals, length of leaf pubescence, and presence or absence of pubescence on sepals and capsules were made from voucher specimens. Seeds produced by cleistogamous and chasmogamous flowers showed no significant differences in morphological characteristics (length, width, weight, colour, and caruncle size); therefore, no distinction was made between them. Data represent means of from 2 to 100 seeds, the total being dependent on the number of seeds produced by each plant cultivated in the greenhouse facilities.

## 6. Scanning Electron Microscopy

A Cambridge Spectroscan 250 was used to make detailed examinations of pubescence and surface characteristics of both seeds and pollen. Samples were glued to the specimen stud using two-sided adhesive tape. The material was treated for four minutes in plasma mode in a sputter coater, shadowing it with approximately 15 nm of gold prior to examination.

## C. Flavonoid Chemistry

### 1. Paper Chromatography

A survey of flavonoids from all field collections and selected herbarium specimens was carried out by extracting 25 mg of dry, powdered leaf tissue in 2 ml of 80% methanol overnight on a shaker. One ml of this extract was spotted dropwise on 46 by 57 cm Whatman 3MM chromatography paper. Each chromatograph was subjected to two dimensional descending liquid chromatography. The first solvent was butanol:acetic acid:water, BAW (4:1:5 by volume, upper phase), and the second was 15% acetic acid. Each chromatogram was dried and rotated 90° between the two solvent runs. The resulting chromatograms were examined for colour under ultra-violet light (3660 Å); in the presence of ammonia vapor and UV light; and, after spraying with NA reagent (diphenylboric acid-2-amino ethyl ester in methanol).



Tissue from five representative populations (D.F. collections 478, 507, 532, 537, and 648) was extracted for identification of constituent flavonoids. Five grams of dried, powdered leaf tissue from each sample were extracted with shaking in 100 ml of 50% methanol overnight, filtered *in vacuo* and re-extracted in the same manner in 80% methanol. The two filtrates from each sample were bulked, and the aqueous phase reduced to a minimum, at 55° C *in vacuo* using a Buchi rotary evaporator.

At least 36 replicates of each extract were spotted and chromatographed in BAW and Acetic acid as previously described. For each extract, only those compounds from the resultant chromatograms which showed a positive colour reaction to NA reagent during the preliminary survey were investigated further.

For each sample, each compound was coded, the spots on the chromatographic paper cut out, and extracted overnight with shaking in 100% methanol. The extracts were filtered *in vacuo* and evaporated to near dryness at 55° *in vacuo* on a rotary evaporator.

Each compound was checked for purity by subjecting it to one-dimensional descending paper chromatography on 23 by 57 cm Whatman 1MM paper in each of four solvent systems: BAW (A); distilled water (B); 15% acetic acid (C); and, 80% phenol in water (by volume)(D) as described in Harborne (1967). Rf values for each compound in each of these four solvent systems were then determined in comparison to Rutin which was run concurrently on each chromatograph. Any further necessary purification was carried out on Whatman 1MM paper using the solvent system which gave the best separation.

Thin layer chromatography (TLC) was also employed on the purified extracts using four different systems. Polyamid-6 (MN Polygram, 0.1 mm thick) plastic TLC plates were run in two separate systems. System E was an aqueous solvent consisting of water: ethanol:methylethyl ketone (70:20:10 by volume) which took about 90 minutes to run 10 cm. The organic solvent (System F), 1,2-dichloroethane: methylethyl ketone:water (50:25:21:4 by volume) took about 65 minutes to run 10 cm. Cellulose plates (DC-fertigplatten PEI-Cellulose) were run in 15% acetic acid for 10 cm, taking about 50 minutes (System G). Silica gel G plates on glass (System H) were run in a solvent system of methylethyl ketone:acetic acid:water (85:12:3, by volume) for 10 cm which took about 45 minutes.



Thus, each extracted and purified compound was run in eight different paper and thin layer chromatographic systems.

## 2. Spectral Analysis

Flavonoids exhibit characteristic absorbance peaks when examined in UV and visible light. The two major absorption peaks occur in the 300-400 nm (Band I) and 240-280 nm (Band II) range and are associated with substitutions on the B- and A- rings, respectively, of the flavonoid structure. A flavonoid compound may be characterized by first determining its absorption spectrum in absolute methanol, and secondly, by the addition of five diagnostic reagents: powdered sodium methoxide ( $\text{NaOMe}$ ), to saturation; 6 drops of methanol saturated with anhydrous aluminum chloride ( $\text{AlCl}_3$ ); 3 drops of 30% hydrochloric acid (HCl); powdered sodium acetate ( $\text{NaOAc}$ ); and, boric acid ( $\text{H}_3\text{BO}_3$ ) as described by Mabry et al., (1970). These spectra were obtained from paper chromatographically purified compounds on a Unicam SP1800 spectrophotometer. Compounds were identified by comparison to published spectral and chromatographic data (Mabry et al., 1970; Jay et al., 1975)

## 3. Hydrolysis of Flavonoid Glycosides

Hydrolysis of the sugar component of the flavonoid glycosides was carried out under acidic conditions. A portion of the purified extract was transferred to a test tube in methanol. A maximum of 5 ml of 10% hydrochloric acid was added, the test tubes were covered with aluminum foil, and heated at  $100^\circ \text{C}$  in a water bath for 90 minutes. On cooling, this mixture was partitioned three times with 2 mls of amyl alcohol, and both fractions were then evaporated to dryness. The alcohol fractions, which contained the aglycones, were redissolved in absolute methanol and subjected to the same spectral analysis described previously.

The acidic aqueous fractions containing the sugar residues were then chromatographed, with standards, on silica gel (DC Fertiplatten S.1 G-25) thin layer chromatography plates. These were chromatographed twice in the same solvent system: ethyl-acetate:isopropanol:water (65:22:11 by volume); then, air dried, sprayed with aniline phthalate reagent (aniline, 4.6 g; Phthalic acid, 8.0 g; n-butanol, 245 ml; ether,



245 ml; and, water, 10 ml), and developed by heating at 100° C for 15 to 30 minutes.

Identification of sugars was accomplished by comparison of Rf values to known standards which were chromatographed at the same time. The most common flavonoid sugar residues, glucose, galactose, rhamnose, xylose and arabinose, were used as standards.

#### D. Numerical Analysis

Fifty chemical and morphological characters were employed in the statistical and numerical analyses (Table 3) including 48 continuous and two classed characters.

Flavonoid characters were scored as quantitative characters on a continuous scale of 0 to 3 where 0 was absent, and 3 indicated a relatively dark spot was present on the given chromatographic paper. All flavonoids reacting positively to NA reagent were included whether or not they were successfully identified.

Chromosome number and seed colour were included as unordered classed characters though it could be argued that they were continuous. By recording these attributes as unordered classes one avoided the problems of *a priori* designations of linear relationships between the character states and of unequal spacing or unequal size of the classes (Sneath and Sokal, 1973).

The Operational Taxonomic Unit (OTU) was defined here as the sum of all individuals observed from an isolated population of plants, including the voucher specimen, living material, and dried material used in chemical analysis. The major criteria for including OTUs in the numerical analysis was the availability of cytological data, though this requirement was waived in the case of type specimens.

The numerical analysis was performed at two levels: on the individual OTUs to investigate the validity of the taxa, and on the taxa for evaluating phenetic and chemical interrelationships. The individual OTU analysis included cytologically known specimens for which flavonoid analysis was available and selected type specimens for which only limited morphological data could be acquired.

The classification program used to aid in establishing taxa was TAXMAP, a program developed by Dr. J. W. Carmichael of the University of Alberta. TAXMAP was employed in preference to the other available classification programs for a number of



**Table 3.** Characters used for numerical analysis.

No.	Type <sup>1</sup>	Character name and states	95% CL	
1	2	K <sup>2</sup> 3-O rut	(0, 1, 2, 3: absent to very dark)	2.00
2	2	A 7-O rut		2.00
3	2	A -		2.00
4	2	K 6-Me,3-O rut		2.00
5	2	-		2.00
6	2	Q 3-O rut		2.00
7	2	Q 3,7-O digly		2.00
8	2	Q 3-O monoglu		2.00
9	2	K 3,7-O rhagal		2.00
10	2	-		2.00
11	2	L 7-O monogal		2.00
12	2	-		2.00
13	2	L aglycone		2.00
14	2	A 7-O monoglu		2.00
15	2	L 7-O monoglu		2.00
16	2	A 6-Me, 7-O ara,glu		2.00
17	2	L 7-O triglu		2.00
18	2	-		2.00
19	2	-		2.00
20	2	-		2.00
21	2	-		2.00
22	2	-		2.00
23	2	-		2.00
24	2	-		2.00
25	2	-		2.00
26	2	-		2.00
27	2	Q 3-O digly		2.00
28	2	-		2.00
29	2	-		2.00
30	5	2N Chromosome number (1,2,3,4: 12,24,36,48)	0.10	
31	2	Cauline leaf length (mm)	4.00	
32	2	Cauline leaf width (mm)	2.00	
33	2	Cauline leaf length / width ratio	0.42	
34	2	Cauline leaf basal angle (°)	16.00	
35	2	Pubescence length (mm)	0.05	
36	2	Margin serration (0, 1, 2, 3: entire to serrate)	0.80	
37	2	Margin ciliation (0, 1: absent, present)	0.80	
38	5	Sepal pubescence (0, 1, 2, 3: absent, auricles, ciliate, all)	0.80	
39	2	Petal length (mm)	0.86	
40	2	Cleistogamous sepal length (mm)	0.42	
41	2	Capsule pubescence (0, 1: absent, present)	0.80	
42	2	Seed length (mm)	0.09	
43	2	Seed width (mm)	0.70	
44	2	Seed length / width ratio	0.12	
45	2	Seed weight (mg)	0.28	
46	2	Caruncle length (mm)	0.10	
47	2	Caruncle length / seed length ratio	0.05	
48	2	Caruncle covering seed apex(0, 1: absent, present)	0.10	
49	6	Seed colour (1,2,3,4,5,6: tan, light brown, brown, dark brown, red brown, white)	0.10	
50	2	Pollen diameter (um)	0.72	

<sup>1</sup>Weighting: 2 = log 2 of number of confidence interval classes in range. Includes continuous state and ordered classes

3 to N - unordered classes; match weight = log 2 N, no-match weight = 1.0

<sup>2</sup>Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside, glu = glucose, rha = rhamnose, gal = galactose, rut = rutin, - = unidentified.



reasons related to the nature of the data used in this study. TAXMAP is versatile in its requirements for data types, it can handle continuous or real number data, ordered classes and unordered classes simultaneously. Distance calculations can incorporate inherent information content provided by the confidence intervals. A very important feature is the ability to handle missing data.

TAXMAP calculates the ranges of the attribute values, normalizes the raw data as fractions of the range, and then calculates a relative distance between each pair of OTUs. TAXMAP also allows for distance calculations to be made by the weighting of attributes according to their relative information content as given by 95% confidence limits. Both equally weighted and weighted analyses were used. The clustering procedure as outlined by Carmichael (1980), was illustrated by means of a taxometric map (Carmichael and Sneath, 1969). Taxometric maps were drawn with the aid of the Calcomp plotter at the University of Alberta Computing Services or by hand based on the information provided by TAXMAP. The map represents a two-dimensional display of the multi-dimensional hyperspace in which the clusters exist.

Three TAXMAP analyses were performed. Firstly, weighted and equally weighted analyses based on all 50 attributes were made of the 59 OTUs representing the author's collections. It was felt that this represented the most reliable analysis based on the greatest available information. A second analysis making use of morphological data only (21 attributes) was compared to the first analysis. The third analysis was run to include a number of herbarium specimens for which cytological data were available and flavonoid analysis was possible, and a number of type specimens for which only limited morphological data were attainable. This brought the total number of OTUs to 95 (Table 4).



**Table 4.** Specimens used in TAXMAP analysis.

OTU Number	OTU Name <sup>1</sup>	Location	2N
1	1-572	Chin Lake, 23km S Taber, Alberta. 3000'.	12
2	4-524	Nose Hill, Calgary, Alberta. 3700'.	12
3	9-550	Little Fish Lake, Alberta. 3600'.	12
4	15-567	Spruce Coulee, Cypress Hills, Alberta. 3600'.	12
5	34-516	Leitch Collieries, Crowsnest Pass, Alberta. 4500'.	12
6	35-517	1km S of Bow Crow Forest Entrance, S of Hillcrest, Alberta. 5500'.	12
7	36-519	Lynx Creek, 19km S of Hillcrest, Alberta. 4500'.	12
8	37-520	Castle River, S of Hillcrest, Alberta. 4500'.	12
9	38-523	Beauvais Lake, Alberta. 4500'.	12
10	54d-533	Nose Hill, Calgary, Alberta. 3700'.	12
11	56-551	Hand Hills, N of Little Fish Lake, Alberta. 3000'.	12
12	57-579	1.8km SW of Cypress Hills Park, Saskatchewan. 4100'.	12
13	58-590	3.7km S of Moose Jaw, Saskatchewan.	12
15	10-556	3.0km E of Cereal, Alberta. 2600'.	12
16	25-545	0.5km W of Turner Valley, Alberta. 4000'.	12
17	22-489	3.6km E of Cawston, British Columbia. 3000'.	12
18	23-487	Courtney Lake, B.C. 3500'.	12
19	24-488	Princeton, B.C. 2500'.	12
20	50-471	57.7km S of Washington state border, Oregon hwy#3.	
21	52-486	11.4km S of Kamloops toward Lac La Jeune, B.C.	12
22	53-507	13.3km E of Grand Forks, B.C. 2000'.	12
23	21-499	Johnston Creek Park, B.C. 3000'.	12
24	6a-570	N bank of Bow River, Scandia, Alberta. 2400'.	24
25	8-538	Cochrane Hill, Cochrane, Alberta. 4000'.	24
26	13-571	N bank of Old Man River, Taber, Alberta. 2500'.	24
27	16a-573	Etzikom Coulee, 38.6km S of Taber, Alberta. 3100'.	24
28	17-576	Writting-on-Stone, Alberta. 3000'.	24
29	54a-537	Nose Hill, Calgary, Alberta. 3700'.	24
30	59-596	3.5km S and 2.3km W of Wood Mountain Park, Saskatchewan.	24
31	26-253	Fabyan, Alberta. 2100'.	24
32	27-531	18km N of Wainwright, Alberta. 2000'.	24
33	62-622	Red Rock Pass, Idaho. 7000'. <b>Type site - erectifolia</b>	48
34	63-631	McDonald Pass, Montana. 6325'.	48
35	64-648	Carthew Summit, Waterton, Alberta. 7500'.	48
36	65-654	Boivin Lake, Alberta. 6800'.	48
37	51-478	1.5km S and 9.6km E of Anatone, Washington.	36
38	40-375	Klickitat Valley, N of Maryhill, Washington.	48
39	41-394	2.7km N and 4.3km W of Lyle, Washington.	36
40	42-396	Trout Lake, Washington. 12,325'.	48
41	43-398	5.8km E of Husum, Washington. <b>Type site - xyloorrhiza</b>	36
42	44-414	Tacoma, Washington. <b>Lectotype site - typica</b>	36
43	45-442	Greensprings Mountain, E of Ashland, Oregon. 4550'.	36
44	46-443	Klamath River, E of Klamath Falls, Oregon. <b>Type site - oregona</b>	36
45	47-458	Klamath Falls, Oregon. <b>Type site - arida</b>	48
46	48-459	Kneeland, California..	36
47	49b-463	Howard's Gulch, 4km NW of hwy#229 on hwy#139, California.	36



48	5b-548	5.7km E of Cluny, Alberta. 2800'.	12
49	7-568	Whitla Coulee, 0.3km E of Etzikom turnoff on hwy#3, Alberta. 2700'.	12
50	12-565	4.6km N of Golden Prairie turnoff on hwy#41, Alberta. 2700'.	12
51	14-566	Medicine Hat, Alberta. 2300'.	12
52	30-547	Bow Crow Forest entrance, 10km W of Turner Valley, Alberta. 4800'.	12
53	31-276	Waterton, Alberta. 4400'.	12
54	29-268	8.0km W of Longview, Alberta. 4300'.	12
55	16b-194	Etkikom Coulee, 38.6km S of Taber, Alberta. 3100'.	12
56	28-256	Porcupine Hills, 8.7km E of hwy#22 towards Nanton, Alberta. 4500'.	12
57	5a-549	5.7km E of Cluny, Alberta. 2800'.	24
58	55-546	Turner Valley, Alberta. 4100'.	24
59	2-112	Expanse Coulee above Old Man River, Alberta. 2500'.	
60	B9634	(MO) <sup>2</sup> Tacoma, Washington. April, 1940.	36
		<b>Lectotype - <i>praemorsa</i> Baker</b>	
61	B8059	(CAS) Humboldt County., California. April, 1935.	36
62	D1738	(MO) Elko Co., Nevada. May, 1969.	48
63	B8385	(DS) Sierra Co., California. June, 1936	48
64	B8403	(DS) Placer Co., California. June, 1936.	48
65	B7354	(CAS, MO) Siskiyou Co., California. April, 1933.	48
66	B8388	(DS) Sierra Co., California. 6770'. June, 1936.	48
67	B5215	(DS) Placer Co., California. May, 1936.	48
68	B8406	(DS) Nevada Co., California. 6000'. June, 1936.	48
69	B8377	(DS) Tehama Co., California. 6000'. June, 1936.	48
70	D1746	(CAS, MO) Owyhee Co., Idaho. May, 1969.	48
71	GD1640	(CAS, MO) Humboldt Co., Nevada. 6960'. June, 1967.	48
72	B8052	(DS) Mount Lassen, California. 5800'. June, 1935.	48
73	B11462	(DS, MO) Klamath River, Oregon. May, 1946.	48
		<b>Isotype - <i>oregona</i> B. &amp; Cl.</b>	
74	B12086	(DS, CAS) Klamath Falls, Oregon. April, 1949.	48
		<b>Isotype - <i>arida</i> B. &amp; Cl.</b>	
75	B7408	(CAS, MO) Kamiah, Idaho. 1500'. April, 1933.	48
76	A8317	(DS) Klamath Co., Oregon.	48
77	B9359	(CAS, MO) Ruby Mountains, Nevada. June, 1939.	12
78	D1754	(MO) Blaine Co., Idaho. May, 1969.	12
79	D1732	(MO) Elko Co., Nevada. May, 1969.	12
80	D1753	(MO) Camas Co., Idaho. May, 1969.	12
81	D1736	(MO) Elko Co., Nevada. May, 1969.	12
82	D1745	(MO) Owyhee Co., Idaho. May, 1969.	12
83	B8699	(DS) Sierra Co., California. 5400'. July, 1937.	12
84	HOOK/K	(KEW)Camas Prairie near source of Columbia. 1834. <b>Type - <i>major</i> Hook.</b>	
85	AN4340	(PH) Pole Creek, Wyoming. May, 1894.	
		<b>Co-type - <i>vall/ico/a</i> Nels.</b>	
86	S10200	(PH) Husum, Washington. June, 1920. <b>Type - <i>xy/orrhiza</i> Suks.</b>	
87	S8530	(PH) Spangle, Washington. May, 1916. <b>Type - <i>subsagittifolia</i> Suks.</b>	
88	CFB67	(DS) Amarron, Colorado. 6900'. 1901. <b>Type - <i>physalodes</i> Greene</b>	
89	RS-BS	(DAO) Duval, Saskatchewan. May, 1942. <b>Type - <i>russelii</i> Boivin</b>	
90	ANSIPH	(PH) Missouri. <b>Type - <i>nutta//ii</i> Pursh</b>	
91	B8662	(MO) 20.5 mi. W of Klamath Falls, Oregon. May, 1937. <b>Type - <i>oregona</i> B. &amp; Cl.</b>	48



92	AN5481	(WS) Continental Divide near Henry's Lake, Idaho. <b>Type</b> - <i>erectifolia</i> Nels.
93	CFB225	(WS, RM) Grand Mesa, Colorado. 9000'. June, 1902. <b>Type</b> - <i>gomphopetala</i> Greene
94	H3156	(WS, DAO, MO, US) Lake Waha, Nez Perce Co., Idaho. 3500-4000'. June, 1896. <b>Type</b> - <i>flavovirens</i> Poll.
95	SMH222	(US, UC, GH) Craig Mountains, Nez Perce Co., Idaho. 900m. May, 1892. <b>Co-type</b> - <i>flavovirens</i> Poll.

<sup>1</sup> Unless otherwise specified, all collection numbers are the author's and consist of a site number-collection number designation. A = E. Applegate, B = M. S. Baker, D = G. Davidse, GD = J. Gentry and G. Davidse, S = W. Suksdorf, AN = A. Nelson, RS-BB = R. S. Russell and B. S. Sallons, HOOK = W. Hooker, CFB = C. F. Baker, H = Heller.

<sup>2</sup> Herbaria from which leaf tissue was obtained for chemical extraction, or where type specimens are located.



### III. Results

#### A. Cytology and Palynology

In his 1964 review, Clausen expressed the need for an extensive cytological investigation of the Chamaemelium in order to provide a more firm basis for the classification of this section. In this study 59 new chromosome counts were made 57 for the Nuttallianae subsection, one in the Purpureae subsection, and one possible hybrid (Table 5). Voucher specimens are deposited at the University of Alberta (ALTA).

Somatic chromosome numbers of  $2N = 12, 24, 36$ , and 48 were found. That these counts are all multiples of six supports the hypothesis that the base chromosome number for the Nuttallianae subsection is  $x = 6$ . When compared to reports of other chromosome numbers in the Chamaemelium the base number for the entire section is likely to be 6 (Davidse, 1976; Clausen, 1964), contrary to the earlier work of Clausen (1927, 1929), which was based on very few counts.

Measurements of pollen size and estimates of pollen viability were made on cytologically known specimens (Figure 2). A t-test indicated no significant difference ( $P = 0.01\%$ ) in pollen diameter or viability estimates for pollen from cleistogamous versus chasmogamous flowers on the same plant (data not shown). All measurements reported here were subsequently made using only pollen from chasmogamous flowers.

The results from stored and fresh pollen measurements were compared for selected samples using the paired sample t-test (Campbell, 1974). At the  $P = 0.1\%$  level, no significant difference was evident in either size or viability, though pollen grains stored in stain tended to be slightly larger than those examined within a few hours of staining (data not shown).

For within-population viability, 2 to 7 measurements from selected populations were examined (data not shown). Standard deviation, SD, for any given measurement was about 9% and the standard error, SE, 2%. Standard deviation, however, could range from 0 to 41% within a population. Within a given sample, pollen size varied about 2.5  $\mu\text{m}$  SD (0.5  $\mu\text{m}$  SE) and within-population variation was only slightly higher (data not shown).



**Table 5.** New chromosome counts in the *Viola nuttallii* complex and other Chamaemelium. All collections were made by the author.

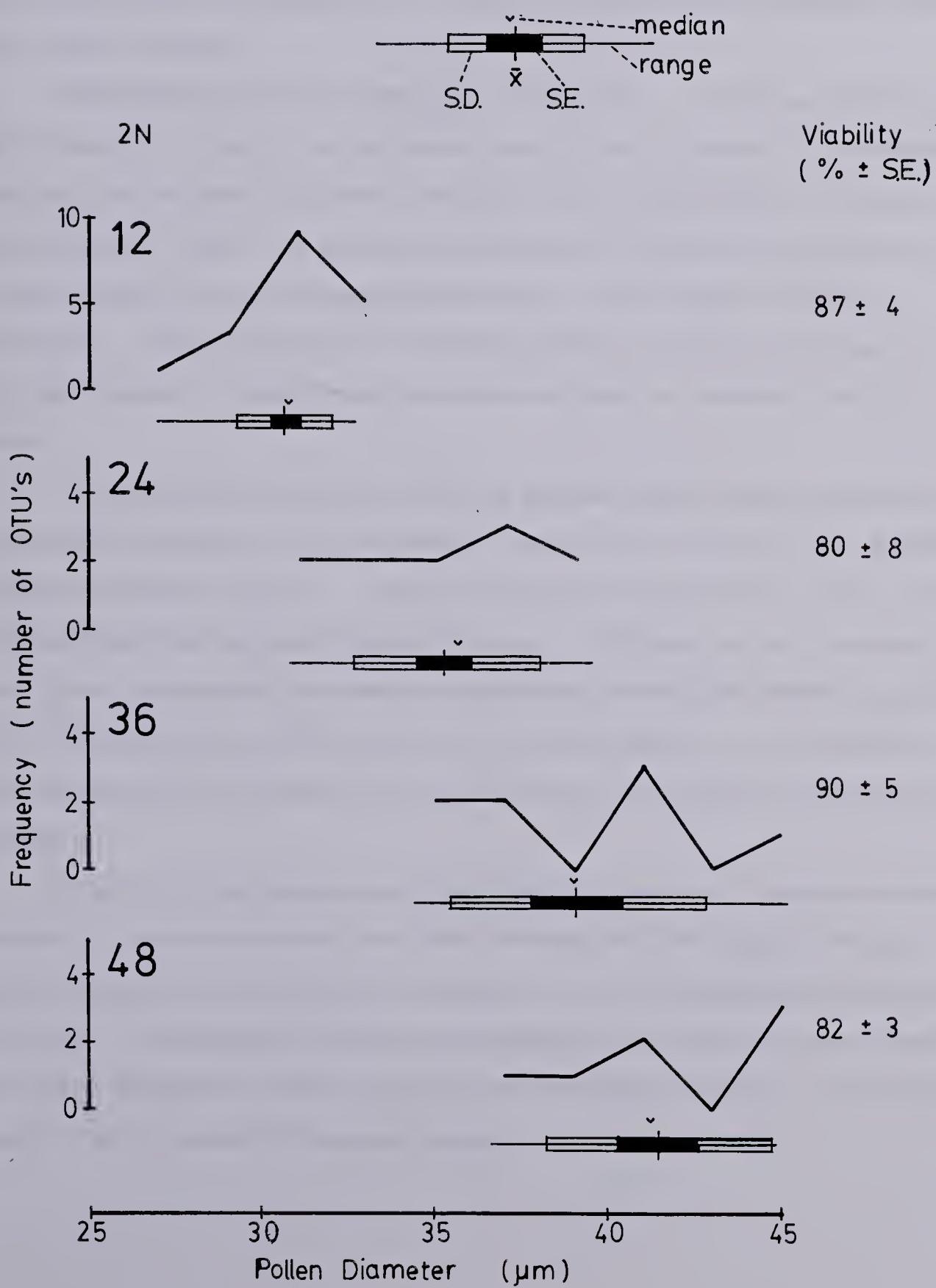
2N	Location	Site Number	Collection Numbers
Subsection Nuttallianae			
<i>Viola vallicola</i>			
12	ALBERTA		
	West bank of Chin Lake	1	105,572
	Nose Hill, Calgary	4	126,524
	Cluny	5b	134,548
	Whitla Coulee	7	148,568
	Little Fish Lake	9	150,550
	Cereal	10	155,556
	Golden Prairie	12	165,565
	Medicine Hat	14	178,566
	Spruce Coulee, Cypress Hills	15	181,567
	Etzikom Coulee	16	194,574
	Turner Valley	25	233,545
	Porcupine Hills	28	256,244
	Bow Crow Forest	30	270,547
	Crownest Pass, Leitch Collieries	34	295,516
	Hillcrest	35	296,517
	Lynx Creek Campground	36	297,519
	Castle River near bridge	37	300,520
	Beavais Lake Park	38	311,523
	Nose Hill, Calgary	54d	532
	Hand Hills NE of Little Fish Lake	56	551
12	BRITISH COLUMBIA		
	Kettle River	20	224
	Johnston Creek Park	21	499
	Cawston Road to Oliver	33	229,489
	Courtney Lake	23	230,487
	West of Princeton	24	232,488
	Lac La Jeune	52	486
	E of Grand Forks	53	507
12	SASKATCHEWAN		
	W of Cypress Hills	57	579
	S of Wood Mountain	58	590
	S of Moose Jaw	60	604
<i>Viola nuttallii</i>			
24	ALBERTA		
	Cluny	5a	128,549
	Scandia	6	145,570
	Cochrane Hill, Cochrane	8	149,538
	Empress	11	161,561
	Taber	13	170,571
	Etzikom Coulee	17	193,573
	Writting-on-Stone Park	17	198,576
	Fabyan	26	253,530
	Wainwright	27	531
	Nose Hill, Calgary	54a	537
	Turner Valley	55	546



24	SASKATCHEWAN Wood Mountain	59	596
<i>Viola praemorsa</i>			
36	WASHINGTON Lyle	41	394
	Husum	43	397, 398, 399
	Tacoma	44	414
	Anatone	51	478
36	OREGON Green Springs Mountain	45	442
	Klamath River	46	443, 444
36	CALIFORNIA Kneeland	48	459
	Howard's Gulch	49B	463
48	WASHINGTON Klickitat Valley north of Maryhill	40	375
48	OREGON Klamath Falls	47	458
48	IDAHO Red Rock Pass	62	622
48	MONTANA McDonald Pass	63	631
48	ALBERTA Carthew Summit, Waterton	64	648
	Bovin Lake	65	654
<i>Viola Bakeri</i>			
48	WASHINGTON Trout Lake	42	395
Subsection Purpureae			
<i>Viola purpurea</i>			
12	CALIFORNIA Howard's Gulch	49A	462
<i>V. purpurea X V. praemorsa</i>			
28	CALIFORNIA Howard's Gulch	49C	462a



Figure 2. Pollen grain diameter and viability estimates for the chromosome races in the *Viola nuttallii* complex.





Pollen viability estimates were generally over 80% for each chromosome class (Figure 2). Very low viability measurements obtained in some cases were likely due to adverse environmental conditions in the field. Where viability estimates were low for pollen samples collected in the field, pollen from the same plant under cultivated conditions typically showed viability greater than 80%. Investigators, therefore, should be wary of basing conclusions regarding hybridization solely on low pollen viability without testing interpopulation measurements and insuring that these measurements are made under "ideal" conditions.

Pollen diameter increased with ploidy level (Figure 2). Pollen size for the two ploidy levels  $2N = 12$  and  $24$  were found to be significantly different ( $P = 1\%$ ) from each other and from the other ploidy levels using the Kruskal-Wallis analysis of variance of ranks (Campbell, 1976). No significant difference ( $P = 1\%$ ) was found between the size of pollen grains in the  $2N = 36$  and  $48$  ploidy levels. Their ranges overlapped significantly. When comparing the frequency graphs, each may be composed of two or three size classes. Conversely, a given pollen size class may represent two ploidy levels.

Scanning Electron Microscopy (SEM) of selected pollen samples confirmed light microscope observations that these medium sized grains were tricolporate with granulate aperture membranes (Figure 3). In the light microscope observations, pollen in polar view appeared to be triangular to circular in shape. SEM observations of untreated pollen grains from voucher specimens revealed elliptic pollen grains as seen in equatorial view, indicating that dry pollen shrinks to a fusiform appearance. The foveolate or pitted surfaces vary in the density and size of pits and in the presence of scabration or rugulation.

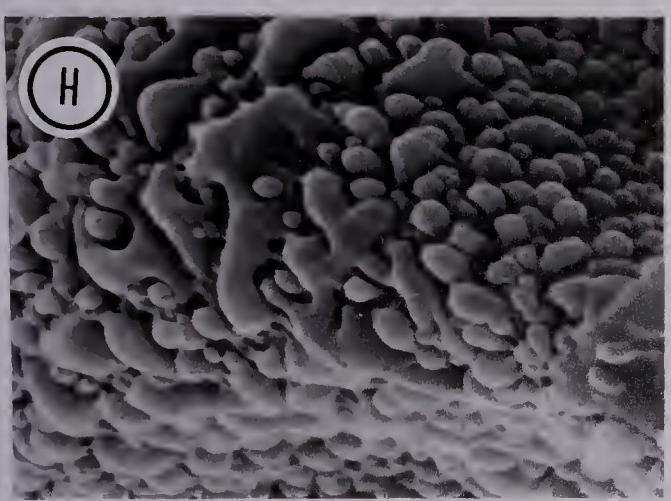
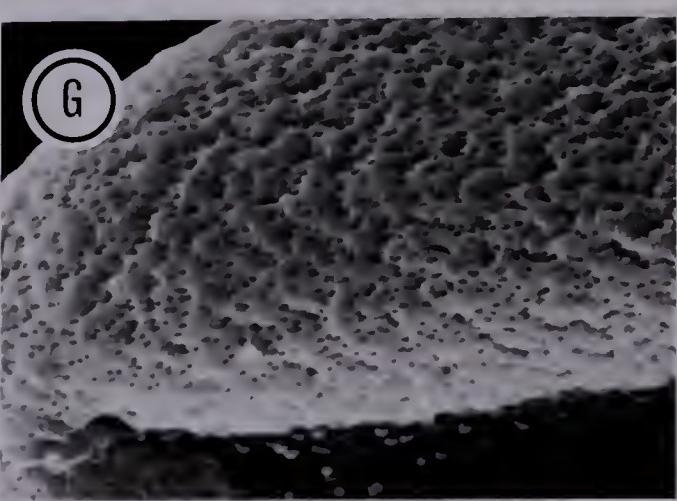
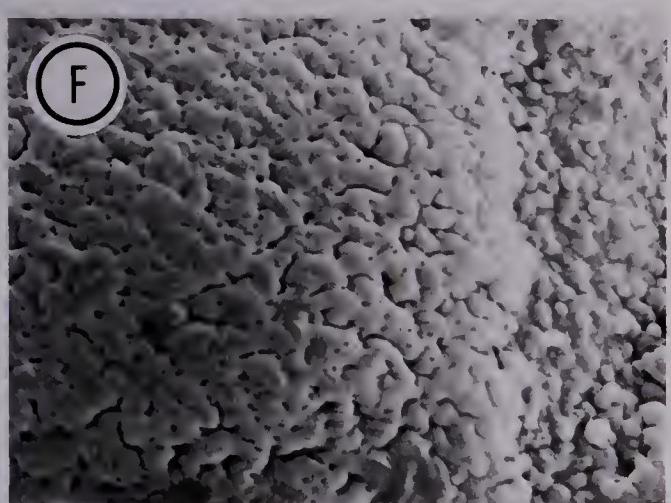
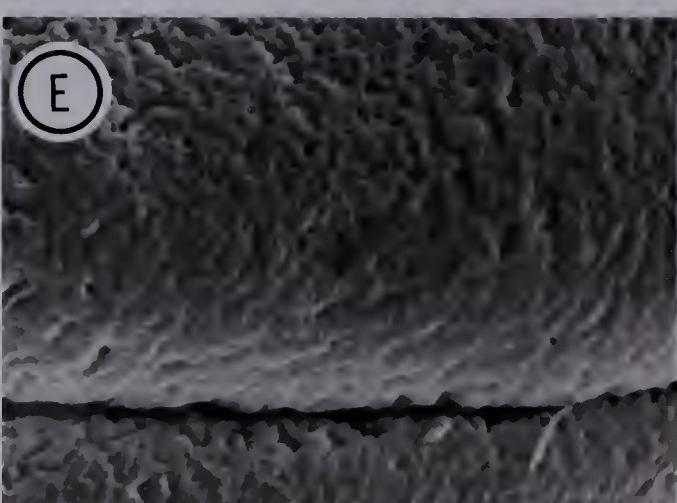
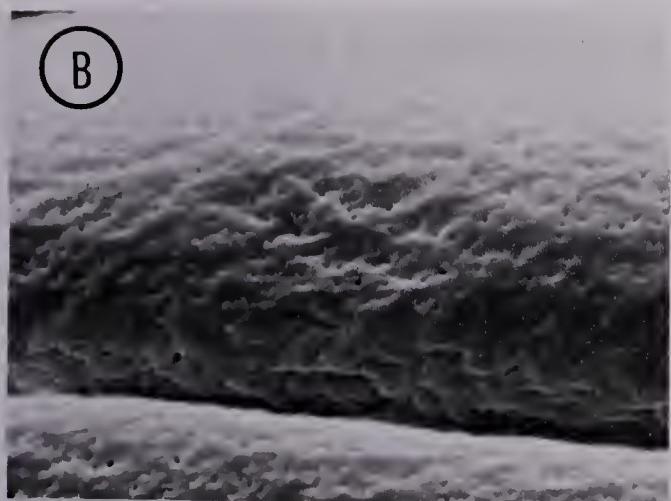
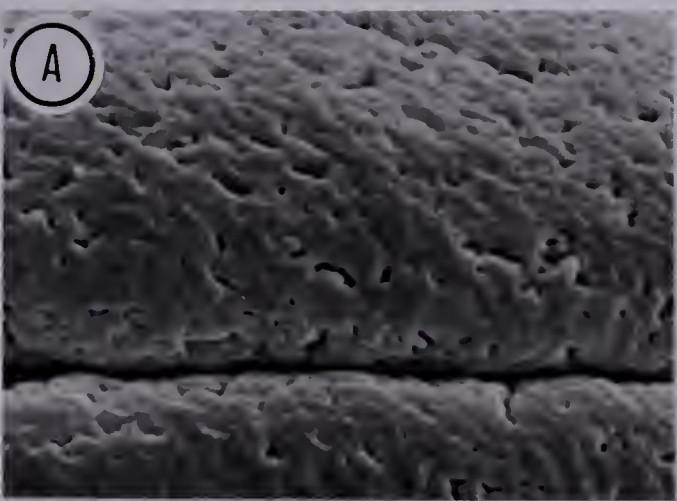
Pollen from cleistogamous and chasmogamous flowers of 15 specimens were examined. In some specimens, some minor differences in pollen grain surface sculpturing were observed between cleistogamous versus chasmogamous flowers (data not shown). No consistant variation was detectable in this limited study due to the small sample size, though there may be a trend toward an increase in the size of scabrae and rugulation with increased chromosome number.





Figure 3. Scanning Electron Microscopy of *Viola nuttallii* complex pollen grains.  
Specimens for each photo are indicated by the site number and collection number; all  
collections were made by the author.

- A. *V. vallicola*, 2N = 12. (DF 50-471)
- B. *V. vallicola*, 2N = 12. (DF 52-486)
- C. *V. nuttallii*, 2N = 24. (DF 8-538)
- D. *V. bakeri*, 2N = 48. (DF 42-396)
- E. *V. praemorsa* subspecies *praemorsa*, 2N = 36. (DF 44-414)
- F. *V. flavovirens*, 2N = 36. (DF 51-478)
- G. *V. praemorsa* subspecies *arida*, 2N = 48. (DF 47-458)
- H. *V. linguaefolia*, 2N = 48. (DF 64-648)





In summary, pollen grain size provided a reliable means of distinguishing two of the ploidy levels and was therefore incorporated into the numerical analysis. Details of pollen surface features were not included in the subsequent numerical analysis. More extensive sampling would be required in order to do this.

## B. Morphology

### 1. Floral Characteristics

Measurements of flower size were carried out on well pressed herbarium specimens and compared to those of fresh flowers. Significant shrinkage occurred in even the best pressed specimens; thus only measurements of live material were included here. Using the total length of the lower, spurred petal as an indicator of chasmogamous flower size (Figure 4), it was evident that, although there was some overlap, there were two distinct classes of petal size. The chasmogamous petal length of the  $2N = 12$  and  $24$  chromosome groups was about  $11.5$  mm, while those of the  $36$  and  $48$  chromosome levels ranged much larger, from  $13.5$  to  $20$  mm long. Thus, this attribute may be useful in distinguishing these two chromosome groups.

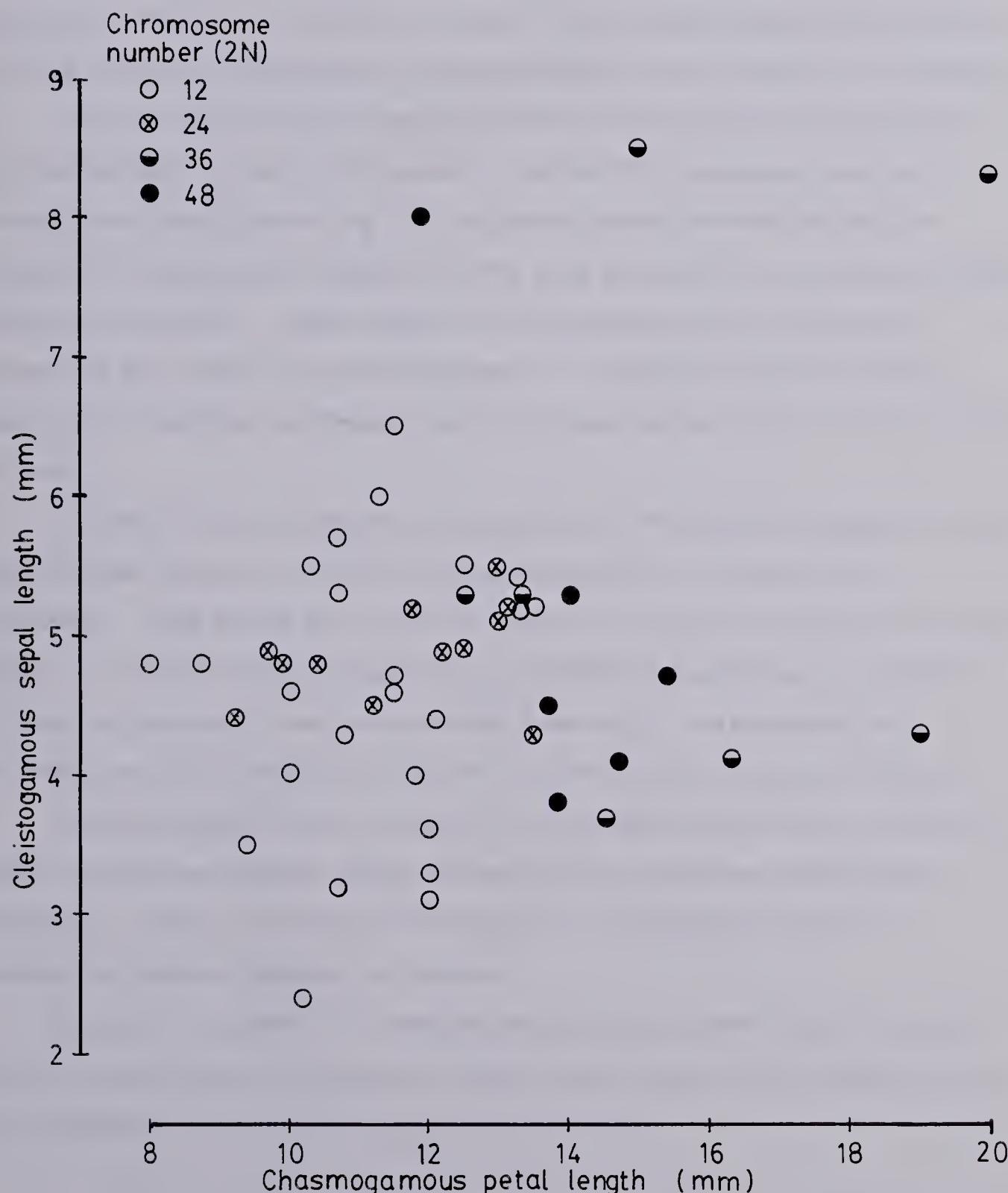
No such reliable separation was evident in the measurement of cleistogamous flower size as measured by the length of sepals (Figure 4). Though little variation occurred within the  $12$  and  $24$  chromosome groups, the sepal length means were not significantly different. A great deal of variation occurred in those specimens with  $36$  and  $48$  chromosome numbers. This attribute was therefore considered to be of limited importance in distinguishing between chromosome groups. It did however, separate out three OTUs which had cleistogamous flowers much longer than the others. This attribute was also included in the numerical analyses.

### 2. Leaf Characteristics

The attributes most commonly enlisted to differentiate among members of the *nuttallii* complex were the characteristics of leaf size and shape: length, width, basal shape of the leaf blade, margin serration, and pubescence. With the exception of serration, these characters were easily quantified. Serration was included in the



Figure 4. Chasmogamous petal length and Cleistogamous sepal length in the *Viola nuttallii* complex.





numerical analyses as an attribute with ordered character states from entire (0) to serrate (3).

Diploid OTUs had generally narrower leaf blades, though their length was not vastly different from those of other OTUs (Figure 5). Tetraploid OTUs, on the other hand, had wider and somewhat shorter leaf blades. As for the octoploids and hexaploids, their leaf blades were wider still and some were much longer than those of the diploids and tetraploids. These two characters, although of obvious value in separating the diploids and tetraploids, were of limited utility in distinguishing between octoploids and hexaploids.

The shape of the leaf blade was described by the two attributes of basal blade angle and the length to width ratio (Figure 6). Diploid OTUs possessed leaves with truncate bases (angles approaching 180°) and blades generally twice as long as wide. Tetraploid OTUs displayed leaf blades up to five times as long as wide with attenuate bases (angles of less than 60°). Again, these two characters alone did not discriminate between the  $2N = 36$  and 48 chromosome levels. These OTUs commonly had leaf blades two to three times as long as broad, with cuneate (angles of 50° to 80°) or truncate leaf bases.

The length of leaf pubescence was measured on trichomes which appeared on the leaf midrib near its base, where even near-glabrous plants often displayed some pubescence. Both groups  $2N = 12$  and 24 had short trichomes averaging 0.14 mm long (Figure 7). The  $2N = 36$  chromosome group exhibited the widest range of variability, from entirely glabrous to those with trichomes 1 mm long. The octoploids had trichomes generally 0.3 mm long, but this fell within the range of hexaploid variability.

Using the Student's t-test (Campbell, 1974), trichome length was not found to be significantly different between diploids and tetraploids, nor between hexaploids and octoploids. There were significant differences ( $P = 1.0\%$ ) between all pairs of combinations between the above two groups.

Because of the ability of this attribute to distinguish between at least two groups of OTUs, it was included in the numerical analysis, even though trichome length alone was not a dependable method of determining ploidy level.



Figure 5. Cauline leaf length and width in the *Viola nuttallii* complex.

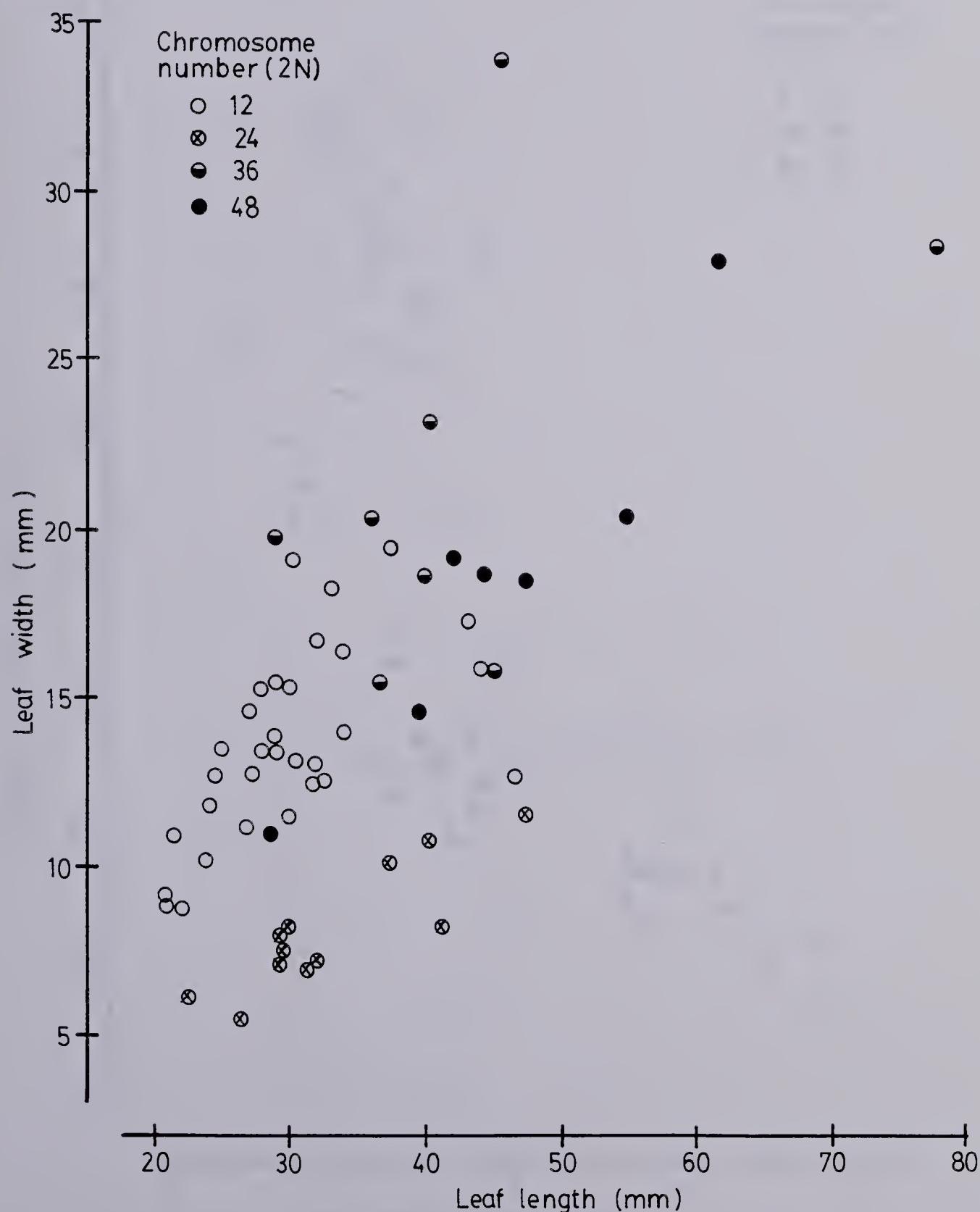




Figure 6. Cauline leaf length to width ratio and blade basal angle in the *Viola nuttallii* complex.

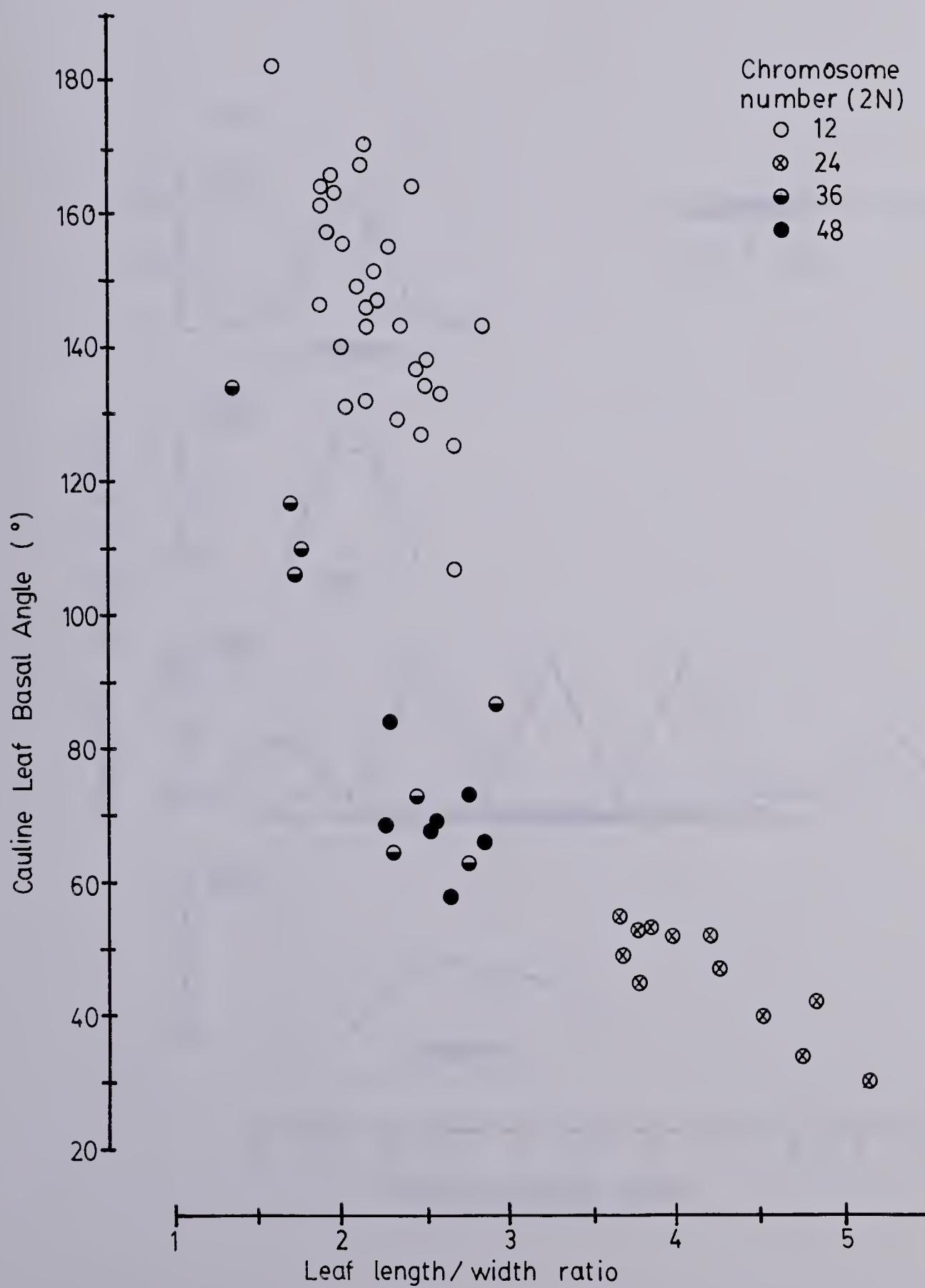
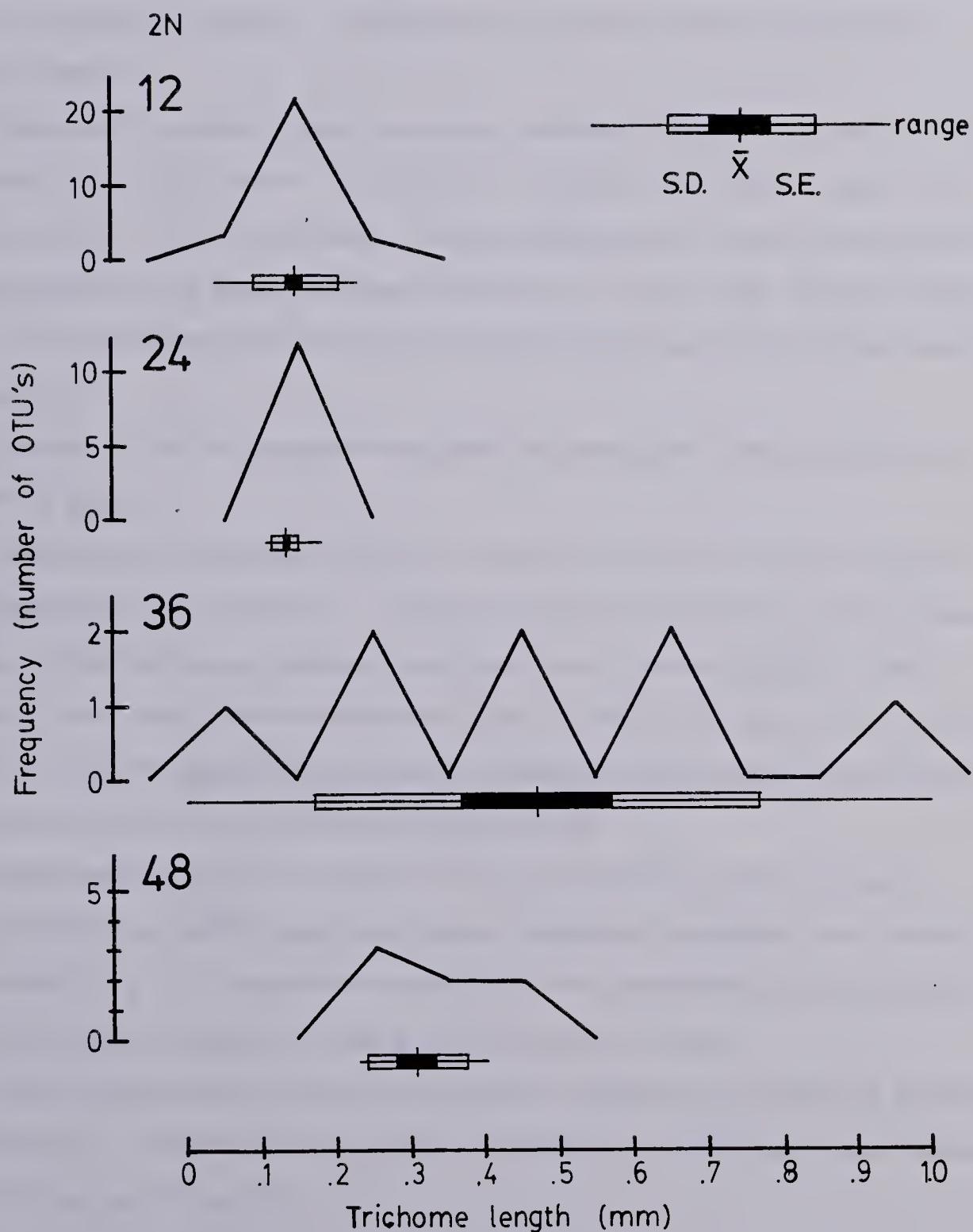




Figure 7. Leaf trichome length in chromosome races of the *Viola nuttallii* complex.





### 3. Seed Characteristics

Size, shape, colour, weight, and caruncle forms of seeds have been described for some taxa in the Nuttallianae (Baker, 1957, 1949b) but have not been used in distinguishing among taxa or groups of taxa.

Seeds were collected from the chasmogamous and cleistogamous fruit of living specimens as described previously. Using the paired sample t-test, no significant differences at the 0.1% level were evident between chasmogamous and cleistogamous seeds for the above attributes. No distinction was made, therefore, between the sources of seeds.

Seed length and width measurements are illustrated in Figure 8 for 58 OTUs, 55 of which were cytologically known. Diploid OTUs possessed tan coloured seeds 1.9 to 2.3 mm long and 1.1 to 1.4 mm wide. Three notable exceptions should be mentioned. All three appeared to be longer than the others and one was also wider (DF 489, 488 and 612). Two of these anomalous OTUs also displayed darker seed colouring than seen in other diploids.

Tetraploid OTUs possessed brown seeds that were both wider and longer than those of the diploids.

Hexaploid OTUs displayed a variety of seed coat colours including white, light brown, dark brown, and red-brown. Hexaploid seeds were normally longer and wider than those of the diploids and generally wider than those of the tetraploids. There appeared to be no clear distinction between hexaploids and octoploids in colour and size of seeds. Figure 8 indicates that diploids and tetraploids can be readily distinguished from each other and from the octoploids and hexaploids.

Seed length-to-width ratio ranges for all cytological types overlap, though tetraploid OTUs have slightly longer seeds and octoploids and hexaploids have generally wider seeds (Figure 9). These differences in form are complemented by differences in weight: seed weight tended to increase with chromosome number.

It was notable that two diploid OTUs (DF 488, 489) displayed characters similar to the tetraploids. These were the same OTUs which showed anomalous character states in seed length and width (Figure 8).



Figure 8. Seed length, width and coat colour in the *Viola nuttallii* complex.

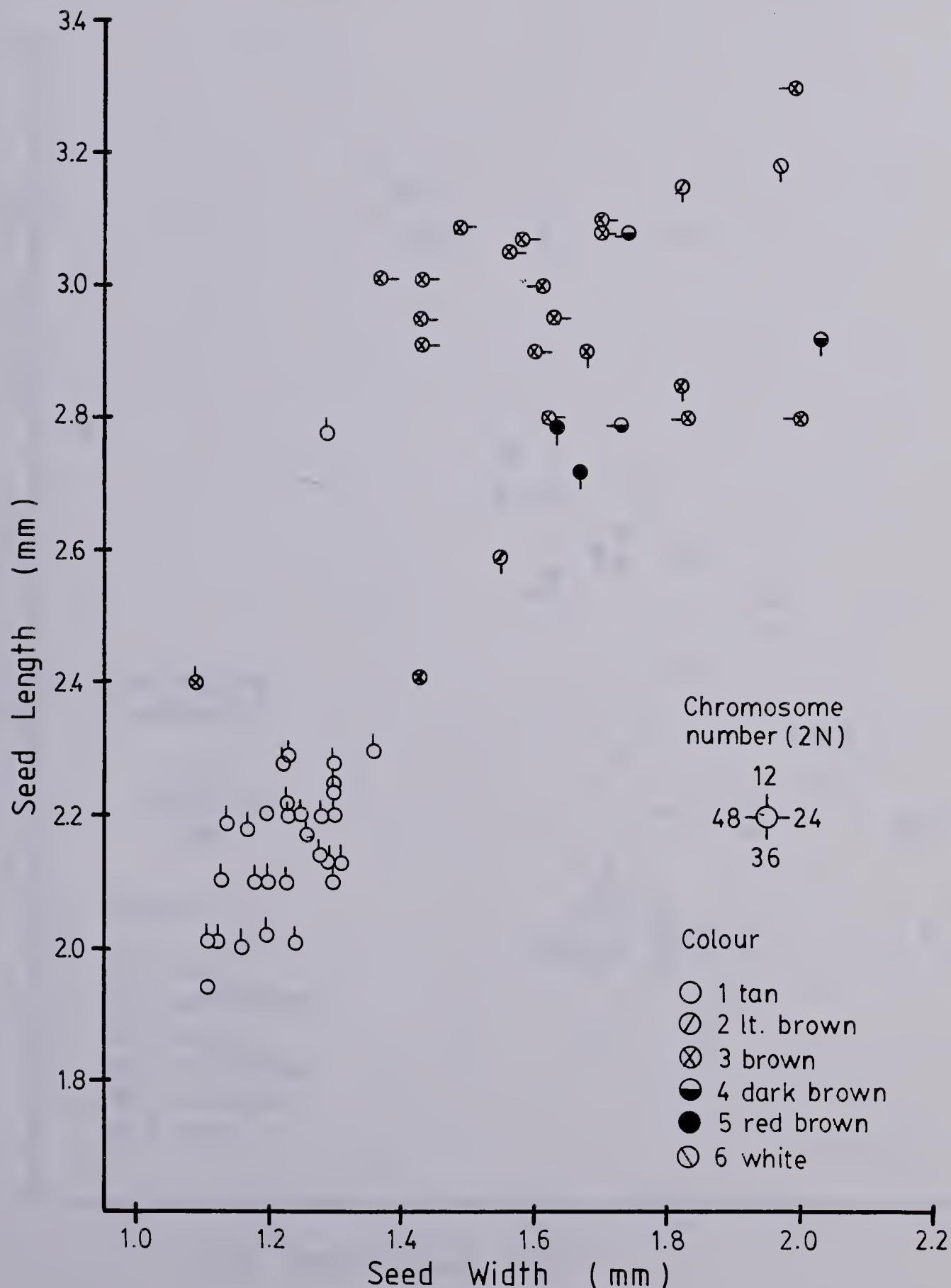
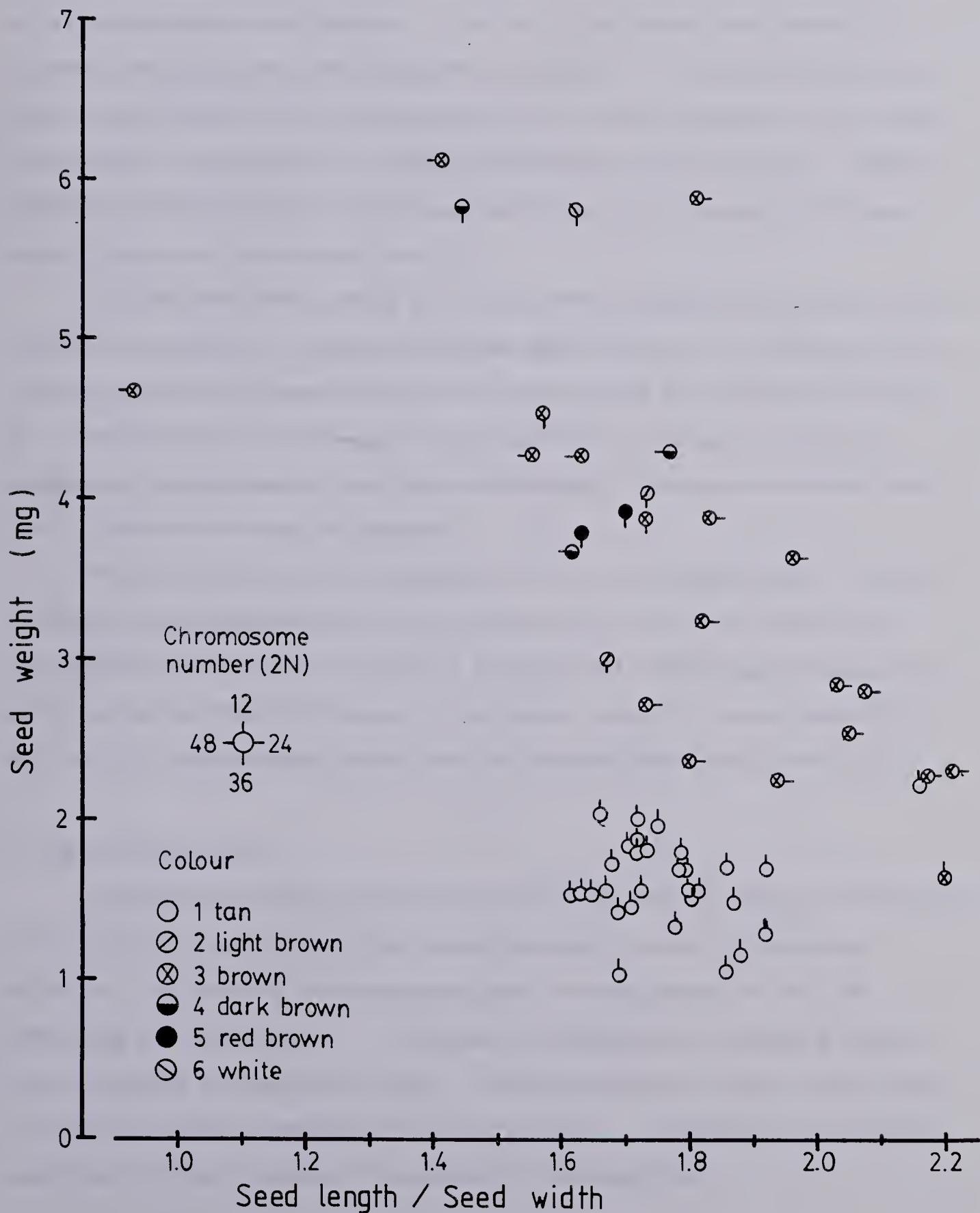




Figure 9. Seed length to width ratio, weight and coat colour in the *Viola nuttallii* complex.





Due to the clear distinction of at least three groups of OTUs by seed length to width ratio, seed weight and seed coat colour, these attributes were incorporated into the numerical analyses.

Baker (1949b) noted that the caruncle of *Viola* seeds was large enough to almost cover the entire funiculus. Through observation, four features of the caruncle seemed to be possible distinguishing features. The form of the caruncle was recorded as unordered state characters, either absent (0) or present (1). The caruncle typically was either dorsally flattened (1) or of amorphous form (0); it either extended to cover the tip of the seed (1) or was attached in a limited area and did not cover the tip (0). These two features and the total length of the caruncle and the percent of the length of the seed which it covered are illustrated in Figure 10.

Diploids possessed caruncles of 0.7 to 0.9 mm in length that extended over 29 to 40% of the seed length. Tetraploids displayed significantly (at  $P = 0.1$ ) longer caruncles which extended over the same proportion of the seed length and often beyond the seed tip. Tetraploid seed caruncle length was not significantly different from either the hexaploid or octoploid seeds, though the relative length of the caruncle was significantly ( $P = 0.1$ ) different from that of hexaploids.

The form of the caruncle correlated well with two cytological groups. The  $2N = 12$  and 24 OTUs possessed seeds with caruncles which were dorsally flattened and covered the tip of the seed, while the  $2N = 36$  and 48 OTUs had globular caruncles which did not extend over the tip of the seed. One notable exception was an octoploid (DF 395) with a dorsally flattened caruncle which did not extend over the tip of the seed.

### C. Flavonoid Chemistry

Twenty-nine flavonoid compounds were detected in the leaf tissue of members of the *Viola nuttallii* complex including flavone glycosides, flavonol glycosides and aglycones. A composite two-dimensional paper chromatographic profile of all compounds is given in Figure 11. All available chromatographic and spectral data for these compounds is presented in Table 6. Compound numbers in Figure 11 and Table 6 correspond to attribute numbers in the numerical analysis. Equivalence of compounds was judged by colour reactions, Rf values and co-chromatography.



Figure 10. Seed caruncle length and form in the *Viola nuttallii* complex.

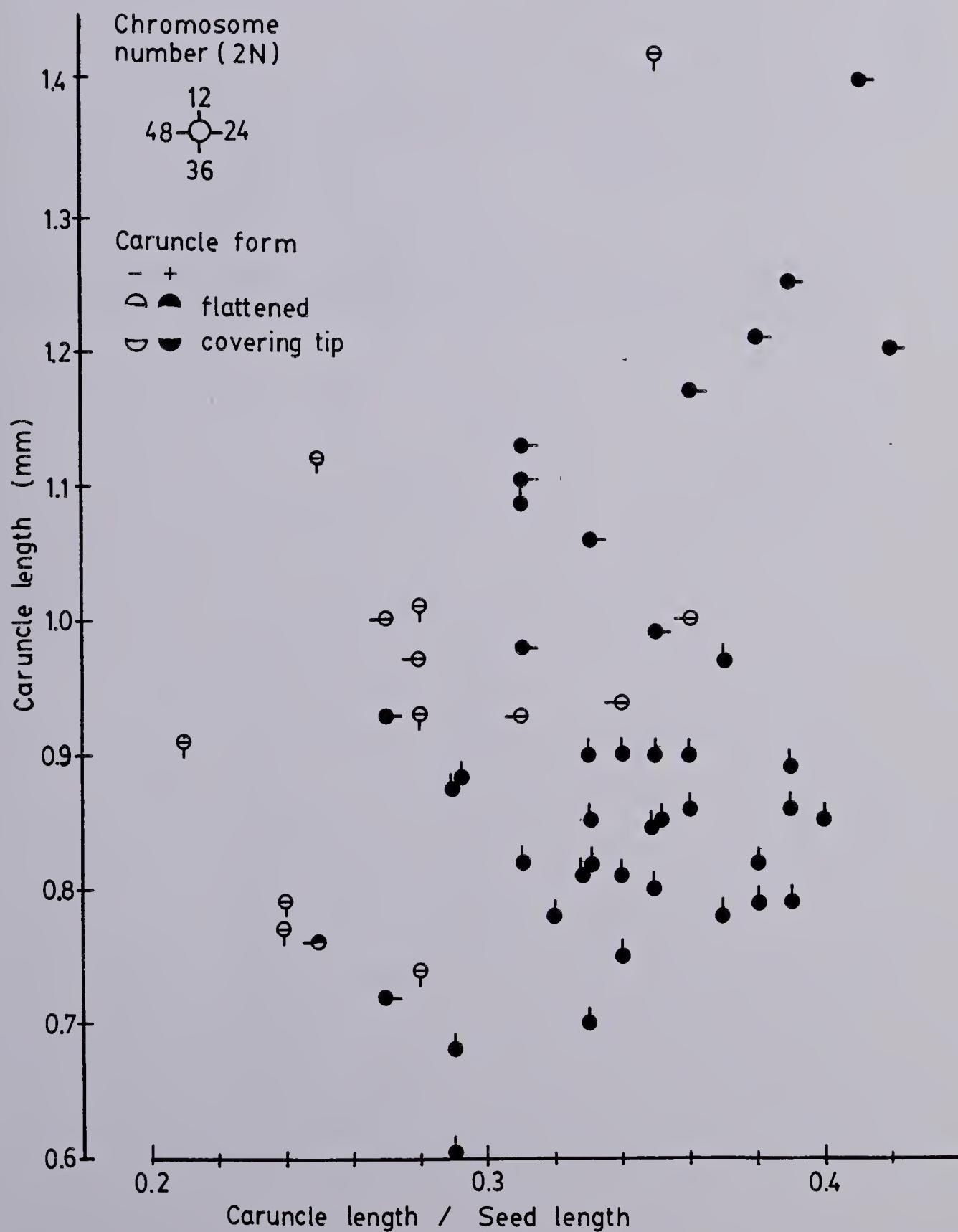






Figure 11. Composite chromatograph of the flavonoid glycosides of the *Viola nuttallii* complex. Numbers correspond to attribute numbers in the numerical analyses.

1. Kaempferol 3-O rut
2. Apigenin 7-O rut
3. Apigenin ?
4. Kaempferol 6-Me, 3-O rhm,glu
6. Quercetin 3-O rut
7. Quercetin 3,7-O diglu
8. Quercetin 3-O glu
9. Kaempferol 3,7-O rhm,gal
11. Luteolin 7-O gal
13. Luteolin
14. Apigenin 7-O glu
15. Luteolin 6-Me, 7-O glu,ara
16. Apigenin 7-O glu
17. Luteolin 7-O triglu
27. Quercetin 3-O digly

Compounds: A= apigenin, K= kaempferol, L= luteolin, Q= quercetin, ara= arabinose, gal= galactose, glu= glucose, gly= glycoside, rhm= rhamnose, rut= rhamnosylglucoside, xyl= xylose.

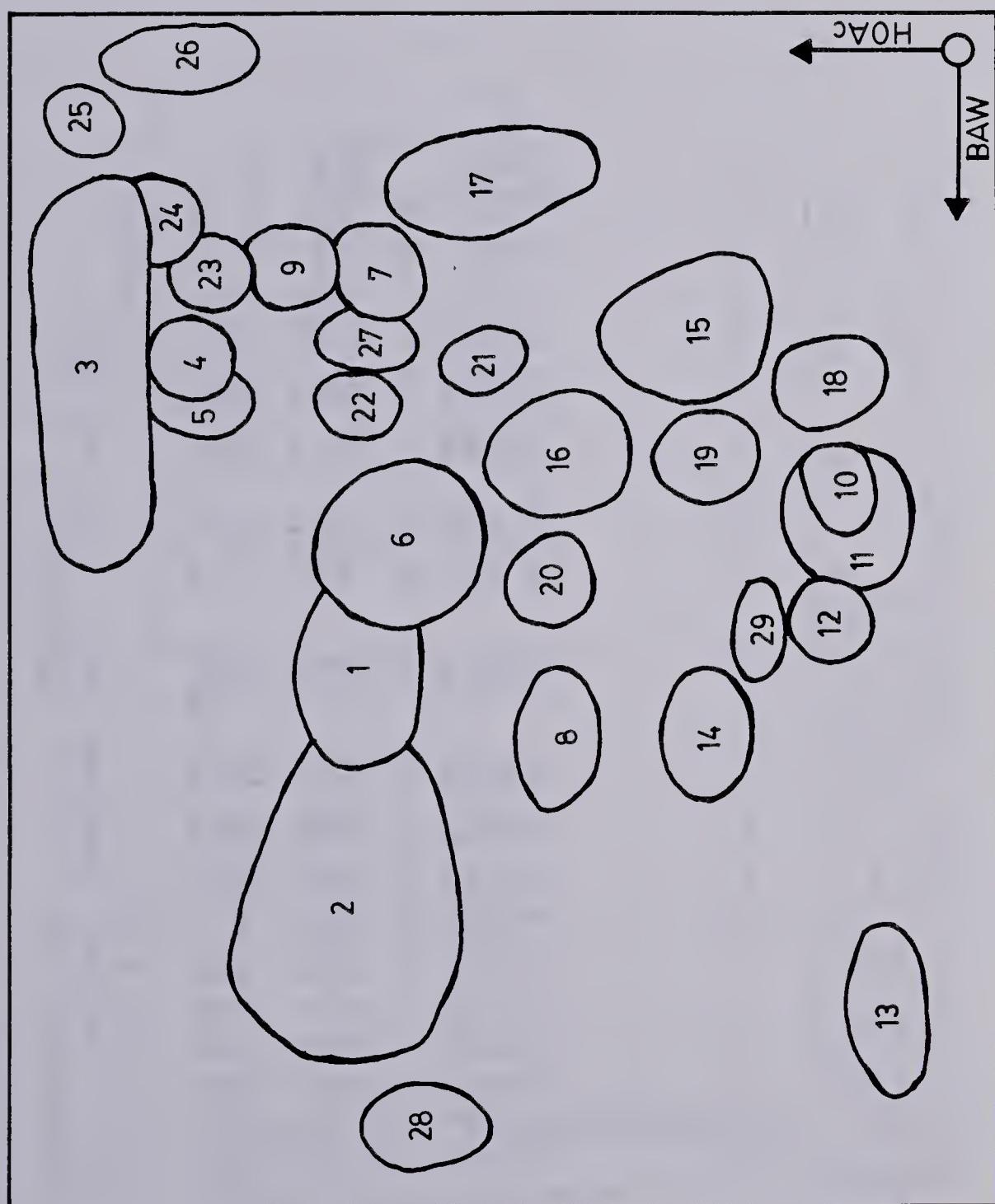




Table 6. Chromatographic and spectral data for flavonoid compounds isolated from the leaf tissue of members of the *Viola nullata*/ii complex.

NO	Colour <sup>1</sup>	Chromatography (Rf × 100)								Spectral Data <sup>2</sup>											
		Paper <sup>2</sup>				Thin Layer <sup>3</sup>				MeOH		NaM		AlCl <sub>3</sub>		+HCl		NaA		H <sub>3</sub> BO <sub>3</sub>	
		A	B	C	D	E	F	G	H	II	III	IV	V	VI	VII	VIII	X	Compound			
1	p-bk-y	58	27	55	64	18	31	60	4	267	349	405	354,402	350,400	405	267	356	K	3-O rut		
2	p-y-yg	71	36	72	70	18	31	60	4	267	349	383	347,402	343,399	382	266	345	A	7-O rut		
3	p-g-yg	33	57	72	55	72	6	89	18	268	335	400	350	347	360	268	345	A	?		
4	p-bk-bk	35	25	61	59					269	328	410	350	348,	399	268	350	K	6-C,3-O rut		
5	p-g-o	36	61																		
6	p-yg-yo	46	23	53	47	25	53	55	260	360	428	435	368,400	405	256	373	Q	3-O rut			
7	p-y-o	24	38	61	40	61	6	69	15	258	360	416	440	366,406	410	262	382	Q	3,7-O diglu		
8	p-y-o	62	5	52	53	6	22	23	82	256	360	430	414	366,400	425	262	382	Q	3-O monoglu		
9	p-g-yg	28	44	69	53	66	6	77	12	266	350	400	352	350	400	266	350	K	3,7-O rhm,gal		
10	o-v-y	32	9	44																	
11	p-y-o	43	1	16	67	11	32	11	81	256	350	398	430	360,390	404	260	374	L	7-O gal		
12	o-o-y	58	15																		
13	p-g-y	86	0	4	59	0	29	4	97	254	348	403	420	358,386	400	252	370	L			
14	p-g-og	58	5	23	67	11	31	16	83	269	329	394	383	344,380	389	269	334	A	7-O monoglu		
15	p-y-yo	29	6	31	51	35	16	27	23	257	351	397	432	366,390	406	260	373	L	7-O monoglu		
16	p-gyg	37	8	39	65	34	31	35	23	23	330	392	349	344	402	269	334	A	6-C,7-O glu,ara		
17	p-y-yo	26	10	38	39	45	5	35	9	258	351	401	433	366,390	403	260	373	L	7-O triglu		
18	p-v-y	33	25																		
19	p-y-y	38	36																		
20	p-g-g	49	47																		
21	p-y-bk	34	58																		
22	p-yg-g	34	69																		
23	p-o-o	24	77																		
24	p-o-o	21	83																		
25	p-o-o	11	88																		
26	p-bl-bl <sub>0</sub>	6	77																		
27	p-yo-o	31	29	60	44	45	10	59	20	258	357	431	406	362,398	410	262	380	Q	3-O digly		
28	p-bl-bl	95	63																		
29	o-w-y	51	15																		

<sup>1</sup> Colours: UV - UV+NH, - UV+NA; bk= black, bl= blue, g= green, o= orange, p= purple, w= white, y= yellow.

<sup>2</sup> Paper Chromatography: A= BAW, B= H<sub>2</sub>O, C= 15% Acetic acid, D= Phenol.

<sup>3</sup> TLC E= Polyamide-aqueous, F= Polyamide-organic, G= Cellulose, H= Silica.

<sup>4</sup> Spectra: 100% MeOH, NaOMe (NaM), AlCl<sub>3</sub>, HCl, NaOAc (NaA), H<sub>3</sub>BO<sub>3</sub>, I = Band I, II = Band II,

<sup>5</sup> Compounds: A= Apigenin, K= Kaempferol, L= Luteolin, O= Quercetin, ara= arabinose, gal= galactose, glu= glucose, rhm= rhamnose, rut= rutin, gly= glycosylation unknown.



Of the 83 OTUs extracted for flavonoid analysis, 79 were cytologically known, 24 of these were specimens taken from herbarium sheets on loan from DS, CAS, UC and MO (Table 4).

Three flavonoid patterns were discovered within the diploid ( $2N = 12$ ) chromosome level (Table 7). Groups 1, 2 and 3, though not morphologically distinct, were distinguished by different chromatographic patterns. In group 1, the major flavonoids 2 and 3 were both apigenin glycosides. Group 3 OTUs contained 1, 9, 10 and 11, kaempferol and luteolin glycosides. Though not consistently separable by means of any of the morphological features examined previously, groups 1 and 3 were found to be geographically separated, occurring east and west of the Rocky Mountains, respectively. Group 2 consisted of six specimens from the Great Basin region, that had intermediate flavonoid profiles. They contained compounds in common with group 1 (1, 2 and 3) and group 2 (1, 5 and 10), and the unique compound, 29.

Group 4, also  $2N = 12$ , consisted of one specimen of *V. tomentosa* (M. S. Baker 8699, OTU 83) which possessed attributes 1, 2, 3, 6, 7, and 8, apigenin, kaempferol and quercetin glycosides.

The OTUs containing 24 somatic chromosomes (group 5) bore some similarity to groups 1, 2, 3 and 4 in possessing attributes 1, 3, 4, 6, and 7. These compounds correspond to an apigenin glycoside, kaempferol 3-O diglycosides and quercetin 3-O and 3,7-O diglycosides.

The eight compounds mentioned thus far were found consistently in over 50% of the OTUs in a given group. The groups contained from 4 to 8 different flavonoid compounds each.

A much greater variety of flavonoid compounds was found in the remaining groups, those with chromosome complements of 36 and 48. These were segregated in Table 7 according to preliminary taxonomic groupings based on identifications made by Baker and Clausen: 6, *praemorsa*; 7, *oregona*; 8, *major*; 9, *arida*; 10, *bakeri*; and, 11, *linguaefolia*.

Group 11 was the only group with a distinct flavonoid profile. Like groups 1 to 5, it possessed compounds 1 and 10; like groups 5 to 10 it contained compounds 6, 7, 8 and 9; and unique to this group was compound 27. Group 11 contained a variety of



**Table 7.** Distribution of flavonoid compounds in the *Viola nuttallii* complex.

Taxon <sup>1</sup> Attribute <sup>2</sup>	Frequency (X100)										
	1	2	3	4	5	6	7	8	9	10	11
2 A 7-O digly	96	17		100							
3 A -	100	100		100	100						
14 A 7-O monogly						100	50	64	40	100	
16 A 6-C,7-O gly						100	100	73	80	86	
13 L						100	50	55	80	86	
11 L 7-O monogly			17			100	100	100	80	100	
15 L 7-O gly						100	100	91	100	100	
17 L 7-O trigly						100	100	73	100	100	
1 K 3-O digly	4	100	100	100	55						100
4 K 3-O digly	13				73						
9 K 3,7-O gly		100				66	100	55	80	86	75
6 Q 3-O digly	4		100	100	100		100	73	100	43	100
8 Q 3-O monogly				100	27	33	50	45		14	50
7 Q 3,7-O gly	9			100	82	66	50	27	80	43	100
27 Q 3-O trigly											75
5 -		100									
10 -		50	17								
12 -					9						
18 -								9			
19 -						33		9	40		
20 -								18	20		
21 -							18			29	
22 -						33	100	18	40	14	
23 -								18		29	
24 -				100				18	40	14	
25 -								45	20	43	
26 -						33	50	9		14	
28 -									60	43	
29 -			16					18			
Total flavonoid attributes/taxon	5	6	4	7	8	13	12	20	15	17	7
Chromosome No.	12	12	12	12	24	36	36,48	36,48	48	48	48
Total populations surveyed	23	6	6	1	11	3	2	11	5	7	4

<sup>1</sup> Taxa: 1 = *vallicola*, 2 = *vallicola*, 3 = *vallicola*, 4 = *tomentosa*, 5 = *nuttallii* 6 = *praemorsa*, 7 = *oregona*, 8 = *major*, 9 = *arida*, 10 = *bakeri*, 11 = *linguaefolia*.

<sup>2</sup> Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside.



flavonols, but no identified flavones: kaempferol 3-O and 3,7-O diglycosides, quercetin 3-O and 3,7-O diglycosides and a quercetin 3-O triglycoside.

Compounds 6, 7, 9, 11, 13, 14, 15, 16 and 17 were commonly found in many of the OTUs in most all of the above groups. These major compounds were apigenin, kaempferol, quercetin and luteolin derivatives. None of the groups, even group 10, *bakeri*, which was morphologically distinct from the others displayed obvious, unique profiles. With increasing chromosome number, OTUs possessed increased numbers of more complex flavonols and flavones.

All raw data presented above and used in the numerical analyses are presented in Appendix 1.

## D. Numerical Analysis

### 1. TAXMAP analysis one

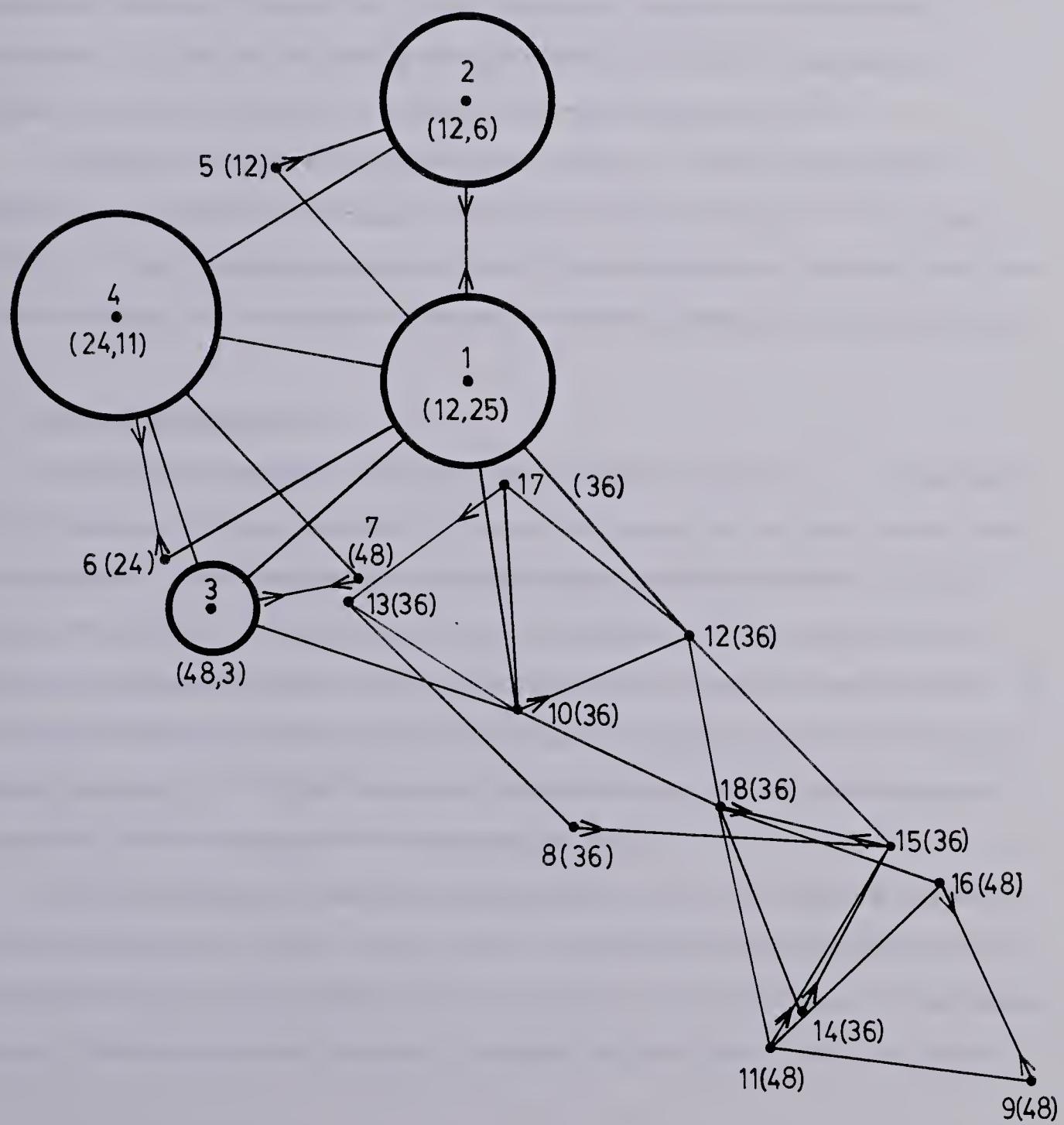
The initial TAXMAP analysis was performed using the author's collections, which totaled 59 OTUs scored for 50 attributes, both structural and chemical in nature. Due to the maximum information content of this analysis, it was presumed to be the most accurate. The resulting clusters from the equally weighted analysis are illustrated in the CALCOMP plot of cluster configurations (Figure 12, see Appendix 2 for the complete TAXMAP printout). This analysis provided 4 multi-membered clusters and 14 single membered clusters.

Cluster 1 included 25 OTUs, all of which were found to have somatic chromosome numbers of 12. All members were located in Alberta and Saskatchewan, east of the Rocky Mountains. The flavonoids present in members of this group were attribute numbers 2 and 3 (apigenin derivatives). These were plants with small (30 mm) ovate or ovate-oblong, nearly entire leaves with truncate bases, covered with very short puberulence, possessing small flowers, and with pollen 31  $\mu\text{m}$  in diameter.

Closely linked to Cluster 1 was Cluster 2 which contained morphologically similar OTUs but which had additional flavonoids: attributes 1 and 9 (kaempferol 3-O diglycoside and kaempferol 3,7-O glycoside, respectively). All six members of Cluster 2 were collected in British Columbia or Oregon, west of the Rocky Mountains.



Figure 12. TAXMAP cluster analysis of 59 OTUs for 50 attributes, equally weighted analysis. Dots indicate cluster centers, circles indicate multi-membered cluster diameters, arrows indicate nearest neighbours, and lines indicate intercluster distances. Each cluster is defined by its cluster number and the chromosome number of its members in parenthesis. For multimembered clusters, the number of OTUs is also indicated in parenthesis.





Cluster 3 consisted of three OTUs possessing flavonoids 1, 6, 7, 8, and 9 (kaempferol and quercetin derivatives). These OTUs had a chromosome count of 48. Their leaves were generally twice as long as wide and about as wide as those in cluster 1 and 2; leaves had more cuneate bases; and, leaf pubescence tended to be slightly longer, about 0.15 mm. Seed morphology also differentiated this group. Cluster 7 (OTU 35) was the mutual nearest neighbour of cluster 3.

Cluster 4 contained 11 OTUs, all having chromosome complements of 24. These OTUs were characterized by their narrow caudine leaves which were more than three times longer than wide and had attenuate bases. Seeds of this group were the largest found in the complex and had brown seed coats. OTUs in cluster 4 commonly possessed flavonoid attributes 3, 4, 6, and 7 (apigenin, kaempferol and quercetin derivatives). Cluster 6 (OTU 29) was a single-membered cluster whose nearest neighbours were all in Cluster 4, and thus it could be considered part of it.

Clusters 8 to 18 were single-membered clusters. None of their nearest neighbours were closer than 0.15 units, unlike the distance relationships within other clusters. A more extensive analysis of these OTUs (analysis three), which included more OTUs in an attempt to overcome the problem of restricted sample size, was performed.

## 2. TAXMAP Analysis Two

Analysis two contained 72 OTUs and is presented in Appendix 3. The additional 13 OTUs represented type specimens (holotypes, paratypes, and isotypes) on loan from other herbaria. Due to the nature of these specimens, limited information could be obtained from them. None of these OTUs were sampled for flavonoid analysis, only three were cytologically known, and none possessed mature seeds for measurement. It must also be recognized when comparing the results of analyses one and two that since distance calculations in TAXMAP represent relative distances, cluster membership was expected to deviate somewhat from that in analysis one.

Whereas analysis one had split violets with  $2N = 12$  into two clusters, analysis two created nine clusters (1, 2, 4, 5, 7, 8, 9, 10, 11, and 26), which were linked or had each other as their eight closest neighbours and very small intervening distances. Membership in some of these clusters was made up of members of both cluster 1 and 2 of analysis



one. For these reasons it seemed most reliable to consider all of these OTUs as one cluster.

The group with  $2N = 12$  now contained four type OTUs: AN4340 (*vallicola* A. Nels.); S8530 (*subsagittifolia* Suks.); RS-BB (*russellii* B. Boivin); and CFB67 (*physalodes* Greene). Single-membered cluster HOOK/K (type specimen of *major* Hooker) had four of its nearest neighbours in this group and was therefore included.

Although the type *major* Hook. clustered here and is the oldest published name associated with this large group, it was not adopted as it has no priority outside the varietal level. Nuttall collected this specimen on the banks of the Columbia River, west of the Rocky Mountains. It was appropriate to apply this name to the chemical form (cluster 2, analysis one) found there. The flavonoid form most common east of the continental divide will be designated *vallicola*, the most eligible and applicable specific name.

Cluster 6 contained OTUs with  $2N = 24$  chromosomes and the same OTUs as cluster 4 of analysis one. Although not a member of it, OTU ANSPH (the type specimen of *nuttallii* Pursh), single member cluster 27, was associated with this cluster by virtue of its nearest neighbour list and its intercluster distances. This specimen is more closely aligned with members of cluster 6 than with any other cluster or OTU. The members of this group shall, therefore, be referred to as *nuttallii*. As no other specimen could be associated with this group, the name is confidently applied.

Cluster 3 of analysis 2 corresponded exactly to cluster 3 of analysis one, containing OTUs of chromosome number 48. It also contained two type OTUs: CFB225, *gomphopetala* Greene and AN5481, *erectifolia* A. Nels. No type specimen of *linguaefolia* Nutt. was available for numerical analysis, but the description and distribution of this taxon indicate that it may be referred here. Close examination of the photographs of the type specimen (PH and G) indicate that based on leaf shape and pubescence as well as its location near the sources of the Oregon [River], it should be included with this cluster. Cluster 3 was assigned the oldest applicable name, *linguaefolia*.

As in analysis one, several OTUs represented single-member clusters.



### 3. TAXMAP Analysis Three

In an attempt to clarify the relationship between the single-membered clusters, a number of additional OTUs from herbarium loans were added to the analysis. These were chosen by virtue of the availability of chromosome counts. Permission was obtained to remove one leaf from each of these specimens for flavonoid extraction. All specimens from clusters 1, 2 and 4 of analysis one, their associated single membered clusters, and associated type specimens were removed from this analysis. Cluster three members were retained for analysis because a number of morphological attributes and chromosome counts of  $2N = 48$  indicated that its members were more similar to the remaining OTUs even though their flavonoid profiles were distinctly different. Analysis three thus contained 39 OTUs, 17 were from the author's collection, 16 from herbarium loans, and eight were type specimens.

The weighted cluster analysis is illustrated in Figure 13 (see Appendix 4 for a complete listing of the TAXMAP analysis). Analysis three produced 10 multi-membered clusters and 9 single-membered clusters.

Cluster one contained the same OTUs as were found in cluster three of analysis two: */inguaefolia*,  $2N = 48$ .

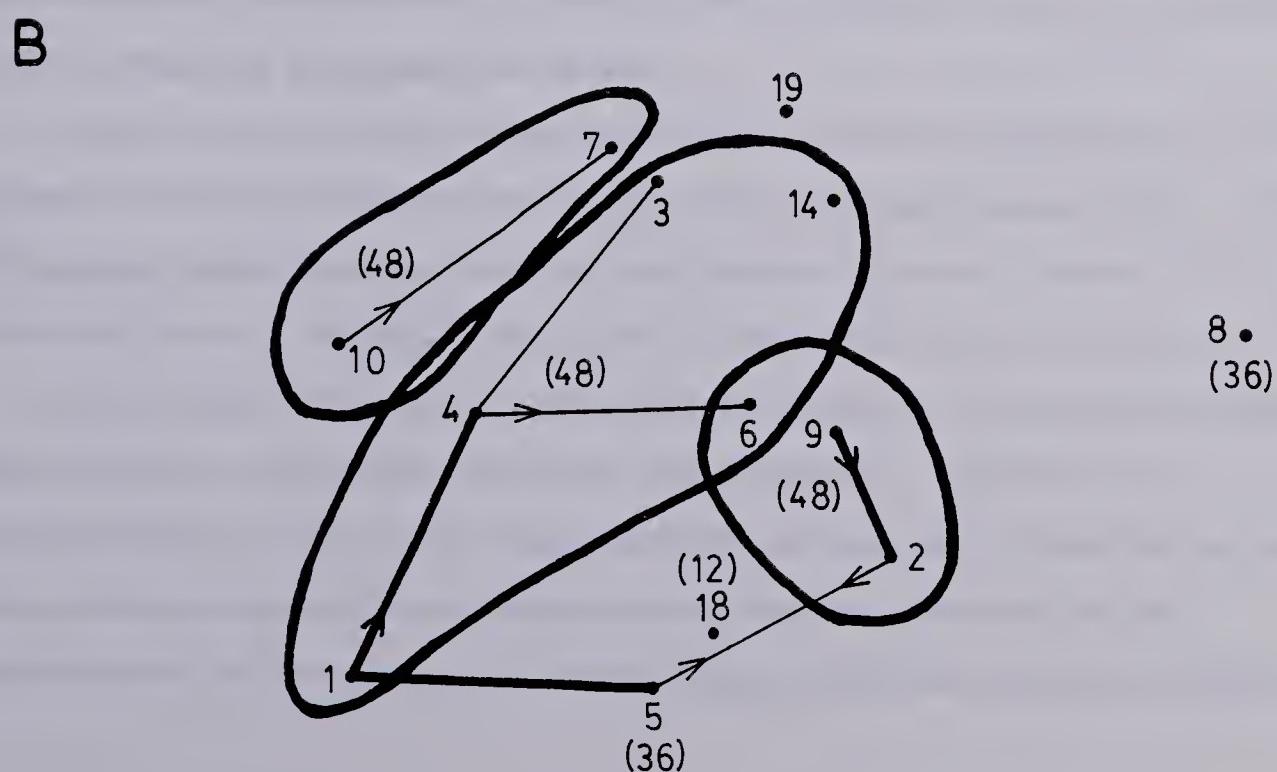
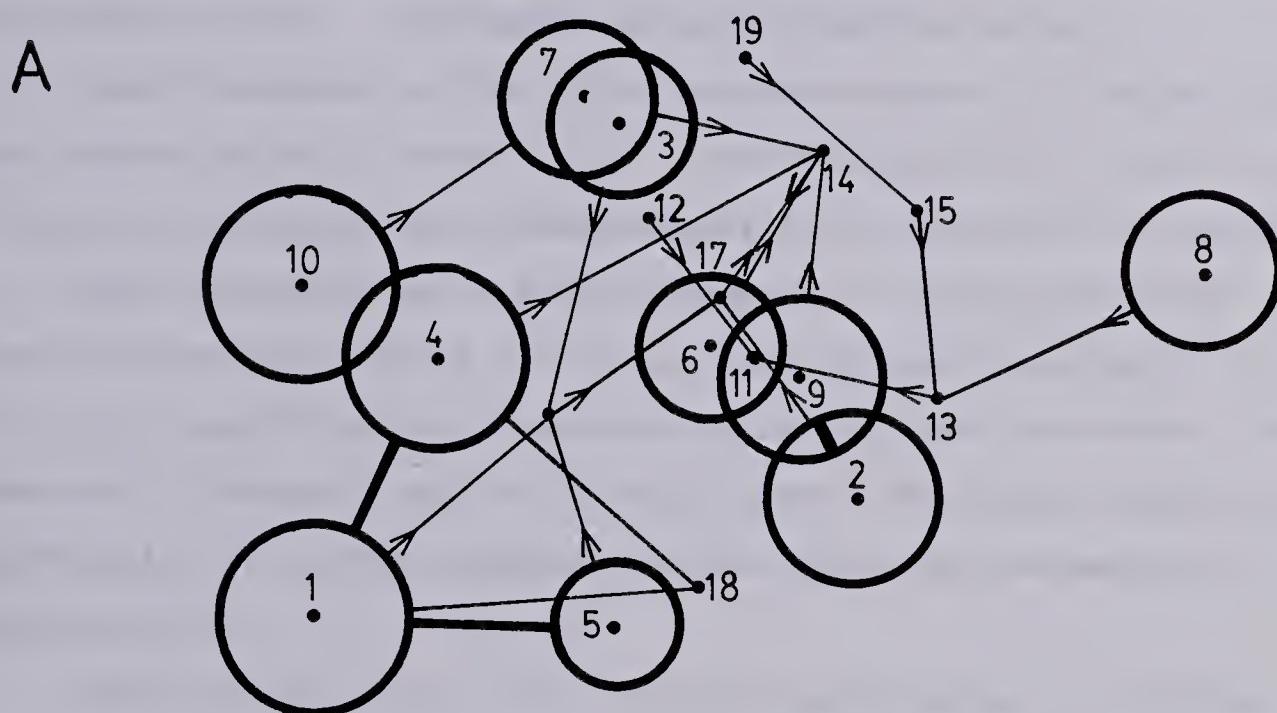
Linked to 1 was cluster 4,  $2N = 48$ , containing the type specimen *oregona* Baker. Cluster 4 differed from one in its lack of flavonoid 1 and the presence of flavonoids 6, 7, 8, 11, 13, 14, 15, 16, 17, and 24 (quercetin, luteolin and apigenin glycosides). These OTUs had shorter leaves only 2.2 times longer than wide, with a basal angle larger than  $90^\circ$ , slightly longer leaf pubescence and some sepal pubescence.

Cluster 5 was also linked to cluster 1, though its members' chromosome complement was  $2N = 36$ . Cluster 5 had no associated type specimen. It differed from clusters 1 and 4 in not containing flavonoids 7, 8 or 24 (quercetin 3-O and 3,7-O glycosides) but otherwise containing all of those found in *oregona*. Leaves in cluster 5 OTUs were slightly longer than wide (ratio of 2.5), had a smaller basal angle than *oregona*, entire margins, no sepal pubescence, seed caruncles were shorter than in */inguaefolia* with red-brown seed coats. In keeping with its chromosome complement of 36, pollen diameter in cluster 5 was smaller than that of */inguaefolia*.



Figure 13. TAXMAP cluster analysis 3, of 79 OTUs for 50 attributes in a weighted analysis.

- A. Calcomp plot of cluster analysis.
  - B. Nearest neighbour analysis with clusters (dots) joined to nearest neighbours (arrows) or linked cluster (heavy lines) and grouped according to TAXMAP analysis using only morphological characters (heavy circles). Cluster numbers correspond to A, chromosome numbers are included in parenthesis.





Cluster 2 members contained flavonoids 7, 9, 11, 13, 14, 15, 16, 17, and 26 (quercetin 3,7-O and kaempferol 3,7-O glycosides), and a variety of luteolin and apigenin 7-O derivatives). These OTUs had small leaves with a length to width ratio of 2.3, a basal angle of 85°, entire margins, chasmogamous flower petals 10 mm long, glabrous capsules, and a chromosome complement of  $2N = 48$ . These OTUs were identified as *Viola bakeri* Greene by M. S. Baker and J. Clausen on annotation labels.

Cluster 9 was linked to cluster 2 in containing basically the same flavonoids and the same chromosome number, but had slightly smaller leaves and larger cleistogamous and chasmogamous flowers. These specimens were also identified as *bakeri*.

Cluster 6 contained two OTUs with chromosome numbers of  $2N = 48$  and with similar flavonoid profiles to clusters 2 and 9. Morphologically similar to clusters 2 and 9, cluster 6 had only slightly larger chasmogamous petals to distinguish it from the others.

Cluster 3 contained the two isotype specimens for *arida* Baker, which have chromosome numbers of  $2N = 48$ . These specimens contained flavonoids 6, 7, 9, 11, 13, 15, 16, 17, and 28 (quercetin, kaempferol, luteolin and apigenin derivatives). Their leaves were 2.0 times as long as wide, 42 mm by 22 mm on the average, a relatively wide basal angle of 110°, very short pubescence, serrulate leaf margins, and sepals with pubescent auricles.

Cluster 7 contained only two OTUs with chromosome numbers of  $2N=48$  and flavonoids 6, 14, 15, 16, 17, and 24 (Quercetin, Apigenin, and Luteolin derivatives). Their ovate-oblong leaves were large, 66 by 30 mm, had cuneate bases, were pubescent, serrate, and had large chasmogamous flowers.

Cluster 7 was the nearest neighbour of cluster 10 which contained the two type specimens of *f/avovirens* Pollard and one OTU with a chromosome count of 36. Most morphological features were very similar though the size of leaves in cluster 10 OTUs were slightly larger. The OTUs in this cluster will be referred to as *f/avovirens*.

Part B of Figure 13 presents a simplification of Part A with most single-membered clusters removed, except those which were type specimens. Clusters were represented only by their centers, linkages between clusters were represented by thick lines, and nearest neighbours were represented by lines with directional arrows. Interpretation of the nearest neighbour index is cautioned as the intercluster distances



represented here were relatively large (greater than .250) and do not vary much at all from intracluster OTU distances.

When TAXMAP analysis three was run using only morphological attributes (30 to 50), the clusters of analysis three were reduced in number (Appendix 4). Figure 13 Part B illustrates the new clusters by circling and linkages between these new clusters was indicated by overlapping the circles. Clusters 1, 3, 4, 6 and 14 were grouped together (*/inguaeolia*, *arida*, *oregona*, *bakeri*, and *arida*, respectively). Clusters 2 and 9 (*bakeri*) were also closely linked to the new larger cluster. Cluster 8 (*praemorsa*) and cluster 5 (triploid) remained intact. Two members of clusters 7 and 10 were clustered together while the other OTUs previously in these clusters were found in single-membered clusters (*flavovirens*).

Cluster 19 (type *xy/orrhiza*) was noteworthy by remaining as a single-membered cluster and not in close association with any other cluster. Cluster 18 (*tomentosa*) also remained as a single-membered cluster, though this was not as surprising considering its distinctive features of chromosome number ( $2N = 12$ ) and very dense tomentose pubescence. This OTU contained the flavonoid constituents 1, 2, 3, 6, 7, 8 and 23 (apigenin, kaempferol, and quercetin derivatives), a range of compounds intermediate to those found in *nuttallii* and */inguaeolia*.

#### 4. Kruskal-Wallis Test

The Kruskal-Wallis test is a nonparametric analysis of rank which does not require the assumptions of normality and equal variances in randomly sampled populations having continuous distributions. This test was employed to test the null hypothesis that the distribution of an attribute was the same in all strata, or clusters, against the alternate hypothesis that at least one population differed from the others.

Table 8 lists all 50 attributes and their significance level based on the Kruskal-Wallis test on eight TAXMAP clusters designated in analysis one and three. The characters were divided into three significance levels. Seventeen attributes including one structural attribute, were not significant at the 5 percent level and were therefore considered invalid for separating taxa. Of the remaining thirty-three attributes, twenty-eight were significant at less than or equal to the 1 percent level. It was attributes in this latter



**Table 8.** Kruskal-Wallis test for significance of flavonoid and morphological attributes.

Not significant at .05 level	Significant at .05 level	Significant at .01 level
<b>Flavonoid Attributes<sup>1</sup></b>		
5 -	14 A 7-O monoglu	1 K 3-O rut
8 Q 3-O glu	16 A 6-C,7-O glu,ara	2 A 7-O rut
10 -		3 A
12 -		4 K 6-C,3-O rut
18 -		6 Q 3-O rut
19 -		7 Q 3,7-O diglu
20 -		9 K 3,7-O rha,glu
21 -		11 L 7-O monogal
22 -		13 L
23 -		15 L 7-O monoglu
24 -		17 L 7-O triglu
25		
26		
27 Q 3-O digly		
28		
29		
<b>Cytological and Morphological Attributes<sup>2</sup></b>		
37 leaf margin ciliation	38 CH sepal pubescence	30 Ploidy level
	40 CL sepal length	31 Cauline leaf length
	41 capsule pubescence	32 Cauline leaf width
		33 Leaf length/ width
		34 Leaf basal angle
		35 Pubescence length
		36 Leaf margin serration
		39 CH petal length
		42 Seed length
		43 Seed width
		44 Seed length/ width
		45 Seed weight
		46 Caruncle length
		47 Caruncle/ seed length
		48 Caruncle covering tip
		49 Seed colour
		50 Pollen diameter

<sup>1</sup> Compounds: A = Apigenin, K = Kaempferol, L = Luteolin, Q = Quercetin, gly = glycoside, ara = arabinose, gal = galactose, glu = glucose, rha = rhamnose, rut = rutin.

<sup>2</sup> CH = chasmogamous, CL = cleistogamous.



group which were considered the most reliable for separating taxa, and were used in making the taxonomic keys.

#### E. Taxonomy

*Viola* L. (Sp. Plant. Ed. I:933. 1753.)

Small trees, shrubs or herbs, the latter annual or perennial; leaves simple, alternate or basal with foliaceous stipules; flowers perfect, zygomorphic, on axillary, one-flowered peduncles, often inverted; chasmogamous flowers, petaliferous, pentamerous and irregular as to calyx, corolla, and stamens; sepals 5, auricled, persistant; petals 5, lowest spurred or saccate, others in two pairs, the laterals usually bearded within, imbricate on the bud; stamens closely surrounding the ovary, distinct but more or less coherent, two lower with nectar bearing appendages projecting into the spur, filaments short and broad or lacking; ovary hypogynous, pistil solitary, style one, with usually oblique stigma, unilocular with three parietal placentae; fruit a loculicidal capsule, ovoid to cylindric, cartilaginous, valves three, seminiferous in middle, contracting when open and ejecting seeds; seeds ovoid, anatropous with a crustaceous coat, carunculate; embryo straight in copious endosperm, cotyledons flat; cleistogamous flowers, if present, with petals rudimentary or lacking, self-fertilizing within the closed calyx, occasionally subterraneous.

Type species of the genus: *Viola odorata* L.

Key to the species of the *Viola nuttallii* complex:

1. a. Leaves entire or nearly so, minutely puberulent, hairs 0.01 to 0.15 mm long, or glabrous; seeds tan or medium brown; caruncle flattened, extending beyond and covering the funiculus.
  2. a. Leaves more than 3.5 times longer than wide, 6 to 13 mm wide, base attenuate (angle 30 to 55°); seeds medium brown, 3mm long and 1.5 mm wide; pollen 35.2 um in diameter; found only east of the Rocky Mountains



- at elevations of 700 to 2400 m; 2N=24 . . . . . *V. nuttallii*
2. b. Leaves 2.0 times longer than wide, base truncate (angle 120 to 180°), some later cauline leaves becoming cuneate; seeds mostly tan, 2.2 mm long and 1.2 mm wide; pollen 31 µm in diameter; found from Great Basin to mid-western plains at elevations of 400 to 2800 m; 2N = 12  
 . . . . . *V. vallicola*
1. b. Leaves entire, serrulate or serrate; pubescent throughout, hairs greater than 0.5 mm long, or occasionally glabrous; seeds silvery-purple to glossy reddish-brown; the caruncle globular, not extending beyond or covering the funiculus.
3. a. Cauline leaves 13.5 mm wide and more 2.3 to 2.5 times longer than wide, margins entire; chasmogamous flowers 8 to 15 mm long; seeds 1.7 to 1.8 mm wide, reddish brown; 2N = 12 or 48
4. a. Densely tomentose throughout, hair length 1.4 mm or longer; seeds 2.7 mm long and 2.0 mm wide, 1.4 times longer than wide, caruncle small, 0.5 mm; 2N = 12 . . . . . *V. tomentosa*
4. b. Densely pubescent throughout, hair length 0.3 to 0.4 mm; seeds 3.1 mm long and 1.8 mm wide, 1.7 times longer than wide, caruncle 0.9 mm long; 2N = 48 . . . . . *V. bakeri*
3. b. Cauline leaves 14 to 30 mm wide and 1.6 to 2.6 times longer than wide, margins crenate or serrate; chasmogamous flowers 12 to 20 mm long; seeds 1.7 to 2.0 mm wide, medium to dark brown; 2N = 36 or 48  
 . . . . . *V. praemorsa*

*Viola nuttallii* Pursh. Fl. Am. Sept., 1: 74. 1814.

Type: "On the banks of the Missouri. June. v.s. Herb. Nuttall." PH!  
 (holotype).

*Crocien nuttallii* (Pursh) Nieuwland and Lunell. Am. Midl. Nat. 4(11): 478. 1916.

A herbaceous perennial, 5 to 20 cm tall, from an erect, woody rootstock; glabrous or with puberulence throughout or only on leaf veins; stem appearing acaulescent



or becoming greatly elongated especially in late season growth; radical leaves ovate to oblong-lanceolate, acute, cuneate to attenuate ; cauline leaves mostly lanceolate, 26 to 40 mm long, 6 to 10 mm wide, 3.5 to 4.5 times as long as broad, some late season exceptions are shorter, base attenuate (angle 40 to 50°); laminal margins entire to distantly subserrulate, minutely ciliate; pubescence about 0.1 mm long; sepals linear, acute, auricles with or without pubescence; corolla yellow, tinted brown outside, lateral petals bearded within , lower and lateral petals marked with brown lines within, lower petal 10 to 13 mm long including short (1 mm) spur; stigma short tubular, style capitate, head retroesely bearded at sides; ovary glabrous or sparsely, minutely puberulent; cleistogamous flowers minute, sepals 5 mm long, common throughout growing season; fruit a valvular capsule producing about 10 seeds per capsule; seeds brown, 3 mm long and 1.5 mm wide, weighing 3 mg each; caruncle distally flattened, 1.1 mm long, extending beyond and completely covering the funiculus; pollen 35.2 um in diameter;  $2N = 24$ .

*V. nuttallii* is widespread east of the Rocky Mountains in the southern regions of Alberta, Saskatchewan and Manitoba, Canada, and in the mid-western United States of Montana, North Dakota, South Dakota, Wyoming, Colorado, Nebraska, and Kansas. It occurs at elevations of 700 to 2400 meters above sea level (Figure 14).

Leaf tissue of specimens from this species was commonly found to contain apigenin, kaempferol, and quercetin flavonoid derivatives.

The specimen designated as the type (Figure 15), "on the sandy plains of the Missouri", appears to be Nuttall's type collection. The plate published in Hooker's *Flora* (1830) has been referred to as the standard drawing of this species (Baker, 1957).

*V. nuttallii* has been found in association with *Phlox hoodii*, *Lithospermum incisum*, *Opuntia polyacantha*, *Bouteloua gracilis*, *Zygadenus elegans*, *Artemisia frigida*, *Allium textile*, *Rhus aromatica*, *Sisyrinchium montanum*, *Cornus alba*, and, *Rosa acicularis*. All of these species are indicative of the xeric conditions to which *V. nuttallii* appears to be adapted. Field observations indicated that, relative to the other members of the Nuttallianae, *V. nuttallii* occurs in the driest conditions.

*Viola nuttallii* has been shown to possess a chromosome number of  $2N = 24$  and shows no evidence of hybridization anywhere within its range. A barrier to gene exchange can reliably be assumed to exist. This taxon is certainly a "good" species



Figure 14. Distribution of *Viola nuttallii* Pursh.

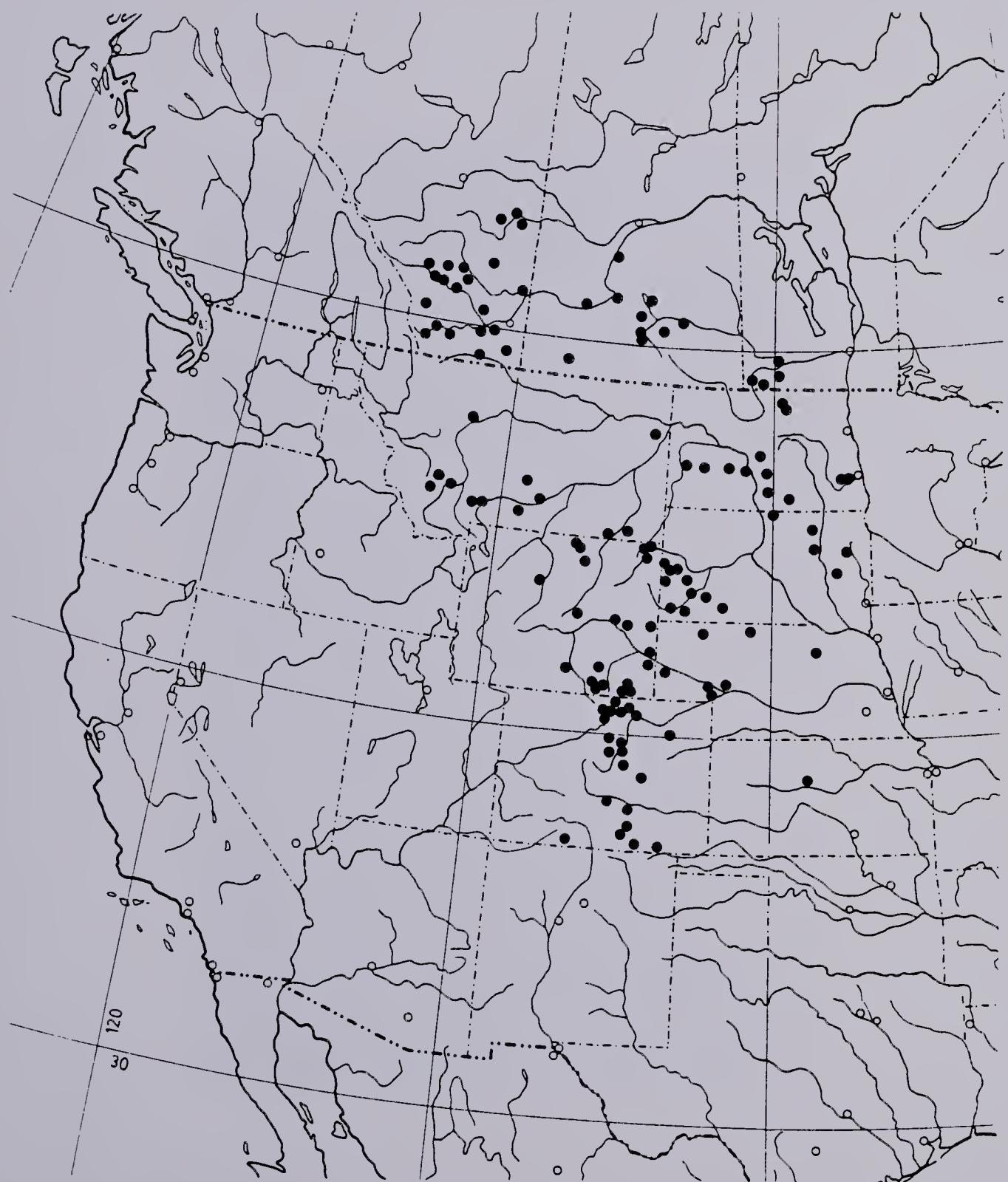






Figure 15. Holotype of *Viola nuttallii* Pursh.



Annotation Label

CENOSPECIES VIOLA NUTTALLII

Viola Nuttallii Pursh

Type - collection!

Det. by M. S. Baker and J. Clausen 1936

ACADEMY OF NATURAL SCIENCES, PHILADELPHIA.

Viola Nuttallii Pursh



according to the definition adopted for this study. Though some variation in leaf shape can be observed, particularly in late season growth, *V. nuttallii* can be reliably distinguished from all other members of the complex by its leaf shape, short puberulence, and large, brown seeds.

*Viola vallicola* A. Nels. Bull. Torrey Bot. Club 26: 122-134. 1899.

Type: "Collected in several places in the state, the following numbers well representing it: 43, 4340, 4345, 4525." A. Nelson 4340, Pine Ridge, Wyoming. June 7, 1898. RM! (holotype).

*Viola glareosa* Dougl. MSS, never published.

*Viola physolodes* Greene. Pl. Baker. 3: 12. 1901.

Type: "In thickets along the Cimarron River, 7 June, 1901." C. F. Baker 67. RM! (isotype).

*Crocien vallicola* (A. Nels.) Nwd. and Lunnell. Am. Midl. Nat. 4(11): 467-487. 1916.

*Viola subsagittifolia* Suks. Werdenda 1: 25-26. 1927.

Type: W. Suksdorf 8530, 10 km southeast of Spangle, Spokane County, [Washington] 26 April and 15 May, 1916. WS! (holotype); PH!, UCI!, MO! (isotypes).

*Viola nuttallii* Pursh variety *vallicola* (A. Nels.) St. John. Fl. S.E. Washington and adjacent Idaho. 1937.

*Viola Russellii* Boivin. Can. Field Nat. 65(1): 22. 1951.

Type: "Saskatchewan: R. C. Russell and B. J. Sallons, Duval, Last Mountain, small draw on hillside. May 13, 1940. DAO! (holotype).

*Viola nuttallii* Pursh subspecies *vallicola* (A. Nels.) Taylor and MacBryde.

Can. J. Bot. 56(2): 190. 1978.

Perennial from relatively shallow-seated rootstock; habit decumbent, subcaulescent, stems elongating and erect late in season; leaves ovate to oblong-ovate, 19 to 43 mm long and 9 to 11 mm wide, blades about 2.2 times as long as broad, truncate to subcordate at base (angle 170 to 185°); glabrous to minutely puberulent throughout; margins entire to subentire, ciliate; chasmogamous flower lower petal 9 to 14 mm long,



sepals glabrous or puberulent on auricles only; cleistogamous flower sepals 4.5 mm long; ovary or expanding capsule glabrous or sparsely, minutely puberulent; seeds 2.2 mm long and 1.2 mm broad, weighing about 1.6 mg each; caruncle distally flattened, 0.8 mm long, extending beyond the funiculus and covering it, extending along up to 40% the length of the seed; seed coat tan with dark brown edges around caruncle; pollen 31.4  $\mu\text{m}$  in diameter;  $2N = 12$ .

*Viola vallicola* has the widest distribution of any taxon within the Nuttallianae, ranging from south-central British Columbia to southwestern Manitoba and the Dakotas, south to Nevada, Utah, Colorado, and Arkansas (Figure 16). It occurs at elevations of 400 to 2800 m. The distribution appears to be continuous across mountain ranges, particularly through the intermountain region of Nevada and Utah. Flowering time was common throughout May though they were not uncommonly observed anytime from late March to early July.

*V. subsagittifolia* Suksdorf from Washington certainly represents *V. vallicola* and must be included here. The new combinations published by St. John (1937) and Taylor and MacBryde (1978) were meant to refer to the taxon located in Washington and British Columbia, respectively. As no specimens were available from which to obtain flavonoid profiles, it was considered more logical to refer these combinations to the specific level. The C. F. Baker specimen from eastern Colorado also must be the eastern chemical form. Though no chemical extractions could be made, it is possible that this collection may be closer to the intermountain intermediate than to the eastern form.

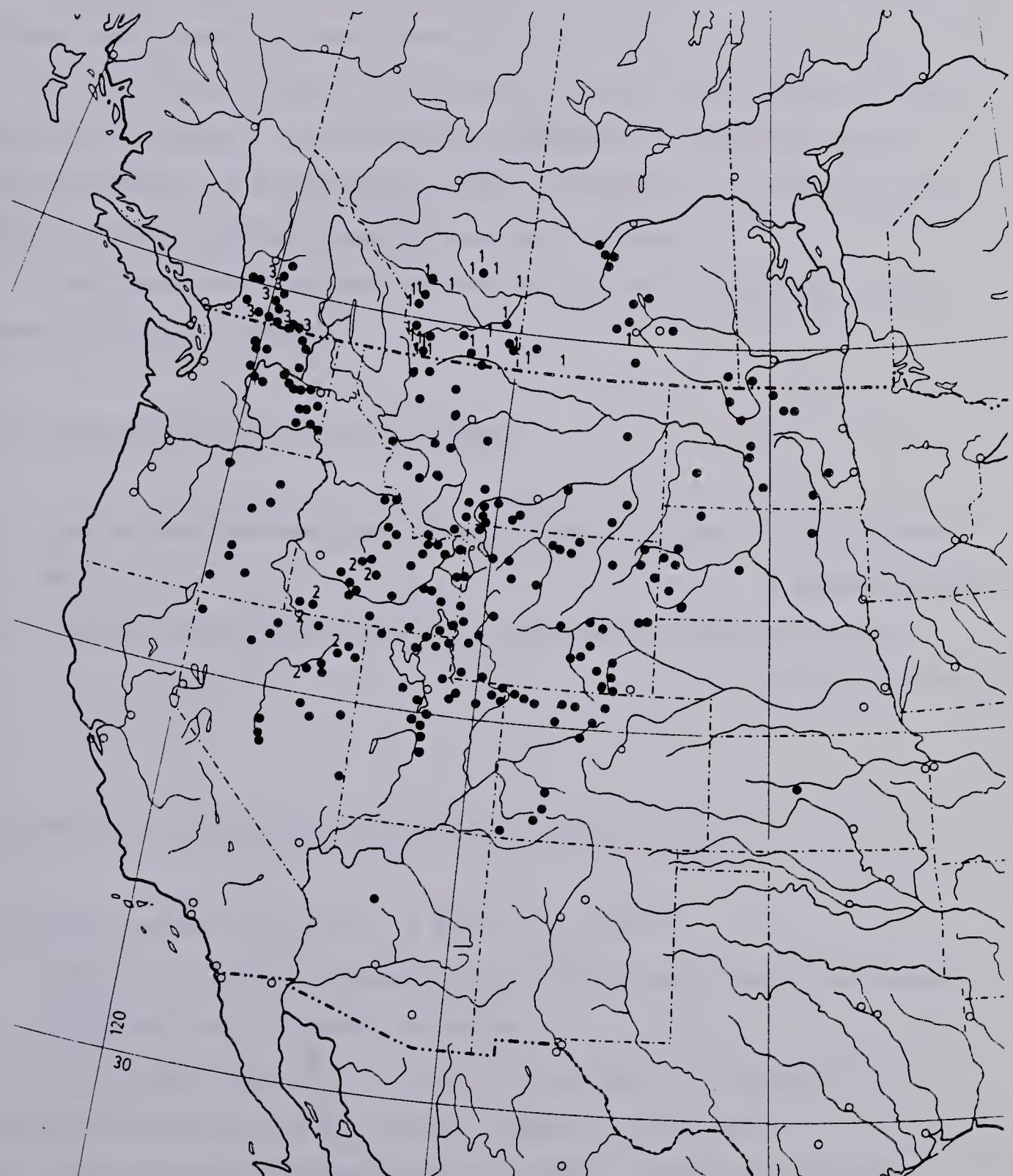
*Crocien vallicola* Nwd. and LII. was recombined as a result of a desire to subdivide the genus *Viola* into several genera based on plant caulescence and type of cleistogamous flowers, if present (Niewland and Kaczmarek, 1914). This division into seven new genera and three subgenera was never widely adopted. The study was based on the examination of very few species, and in fact did not include any members of the *nuttallii* complex. The section and subsection notation as used in this study was much more widely accepted and used since it was thought to reflect *Viola* as a natural group.

Boivin's description of *V. russepii* (1951) represents a species whose attributes match those of *vallicola* and whose type was located in the range of the eastern subspecies, *vallicola*. The publication gives no explanation for segregating *russelii*



Figure 16. Distribution of *Viola vallicola* Nels.

Black dots represent specimens of *V. vallicola*, 1= subspecies *vallicola*, 2= flavonoid intermediates, and 3= subspecies *major*.





and *vallicola* though in a reply to an inquiry from M. Bowerman (1952, see DAO 288885) Boivin makes it apparent that no type specimens were examined and that he found it "difficult . . . to state the differences between these two species." Listed paratypes were from British Columbia, Alberta, and Montana, and the type was from Saskatchewan. This taxon was considered as synonymous with *V. vallicola*.

*V. vallicola* does display some variability in the size of leaves, degree of basal truncation, stem elongation, and chasmogamous flower size. Despite these minor variations, specimens of this species can easily be distinguished from *nuttallii* by leaf shape, and from other Nuttallianae by their near glabrous leaves.

Two chemotypes were observed within this species which likely represent two extremes in a range of variability.

Key to the subspecies of *Viola vallicola* A. Nels.

1. a. Leaf flavonoids primarily kaempferol derivatives; found west of the continental divide . . . . . subspecies *major*
1. b. Leaf flavonoids all apigenin derivatives; found east of the Rocky Mountains . . . . . subspecies *vallicola*

***Viola vallicola* A. Nels. subspecies *major* (Hook.) Fabijan comb. nov.**

*Viola nuttallii* variety *major* Hook. Fl. Bor. Am. 70. 1830.

Type: "Abundant under to shade of pines on the dry sandy plains of the Columbia." KEW! (holotype), UC! (photo of holotype).

*Viola nuttallii* *major* (Hook.) Piper. Contr. U. S. Nat. Herb. 11: 393. 1906.

*Viola praemorsa* variety *major* (Hook.) Peck. Madrono 6: 135. 1941.

*Viola praemorsa* Dougl. subspecies *major* (Hook.) Baker. Madrono 10:128. 1949.

Leaf flavonoids primarily kaempferol derivatives; occurring west of the continental divide.



Subspecies *major* occurs west of the Rocky Mountains in British Columbia and Oregon (number 3 in Figure 16).

William Hooker (1830) described *major* as having wider leaves and larger flowers than *V. nuttallii*. The only specimen referred to was a Douglas collection which he had described in manuscript as a new species, *V. glareosa*. This specimen (H 1526/82) was from Herbariorum Hookeriana, was designated as the type at Kew (Figure 17). The label reads "*Viola glareosa* abundant under shade of solitary pines on dry sandy soil of Columbia." There can be little doubt of its application to the epithet "*major*", and thus, its connection to the western flavonoid form of *V. vallicola*.

#### *Viola vallicola* A. Nels. subspecies *vallicola*

*Viola vallicola* A. Nelson. Bull. Torrey Bot. Club 26: 122-134. 1899.

Type: "Collected in several places in the state, the following numbers well representing it: 43, 4340, 4345, 4525." A. Nelson 4340, Pine Ridge, Wyoming. June 7, 1898. RM! (holotype).

Leaf flavonoids all only apigenin flavones; occurs east of the continental divide in Alberta and Saskatchewan.

Though there was a tendency to shorter, wider cauline leaves, the range of variation was too great to be significant. The only reliable means by which to make identifications being geographical, it was thought that distinction at subspecies level was acceptable between *major* and *vallicola*.

The specimens cited by Nelson (1899) were all collected in Wyoming. Though number 43 was the first number referred to in publication, number 4340 was designated as the type specimen in the Rocky Mountain Herbarium. Pine Ridge is in the Black Hills in Crook County, northeastern Wyoming and logically may be referred to the eastern flavonoid form.

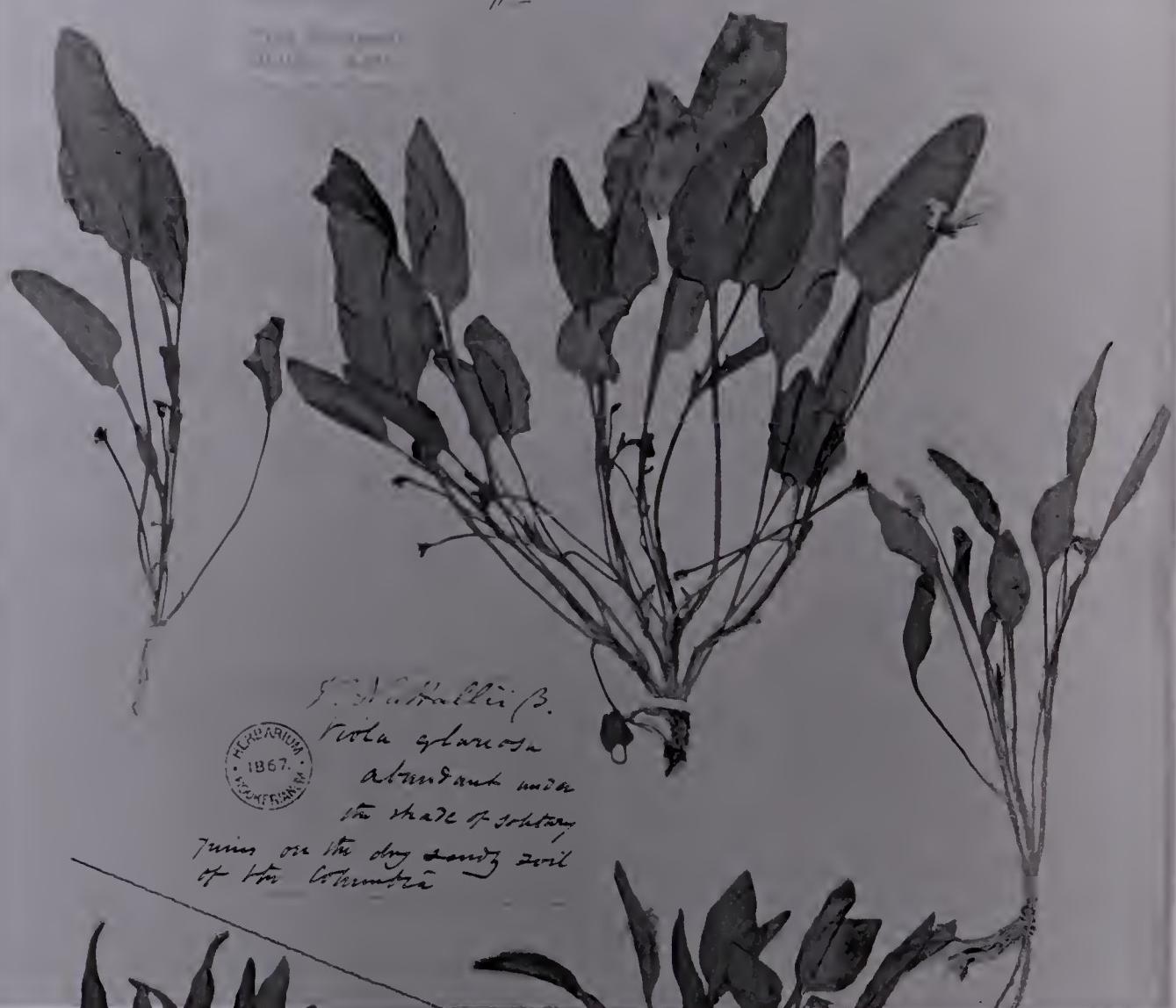
The two chemical forms, *major* and *vallicola*, could not be reliably distinguished on the basis of any morphological character examined in this study. Although there was a tendency for *major* specimens to display slightly smaller leaf length-to-width ratios, and





Figure 17. Holotype of *Viola vallicola* A. Nels. subspecies *major* (Hook.) Fabijan.

*Kola Nuttallii* var. *major* Hook. & Arn. Amer. J. 29 (1830),  
Type.





subentire, ciliate leaf margins as compared to *vallicola*. As noted in the morphological results, two specimens of *major* showed longer or darker seeds. None of these attributes were consistent or significant.

The only reliable means of identifying the subspecies appeared to be flavonoid chemistry, with apigenins commonly found in *vallicola* and kaempferols in *major*. These two taxa are not geographically isolated except in the northern extreme of the range. Genetic isolation cannot be assumed between the subspecies which likely intermingle, particularly in the intermountain regions of Idaho and Nevada. Examination of the leaf flavonoids from specimens in that area (OTUs 77, 78, 79, 80, 81; B9354, D1754, D1732, D1753, and D1736, respectively) contained apigenin and kaempferol derivatives.

*Viola tomentosa* Baker and Clausen. Leafl. West. Bot. 5: 141-147. 1949.

Type: *M. S. Baker* 8722A, "July 9, 1937 (early growth), . . . collected along California highway 20, about 13 miles westerly from Cisco, Nevada Co., California, at about 5000 ft. elevation in forests of *Pinus ponderosa*, *Quercus Kelloggii*, and *Libocedrus decurrens*." CAS (holotype), UCI, OSU, MO, NY, US, and G (isotypes).

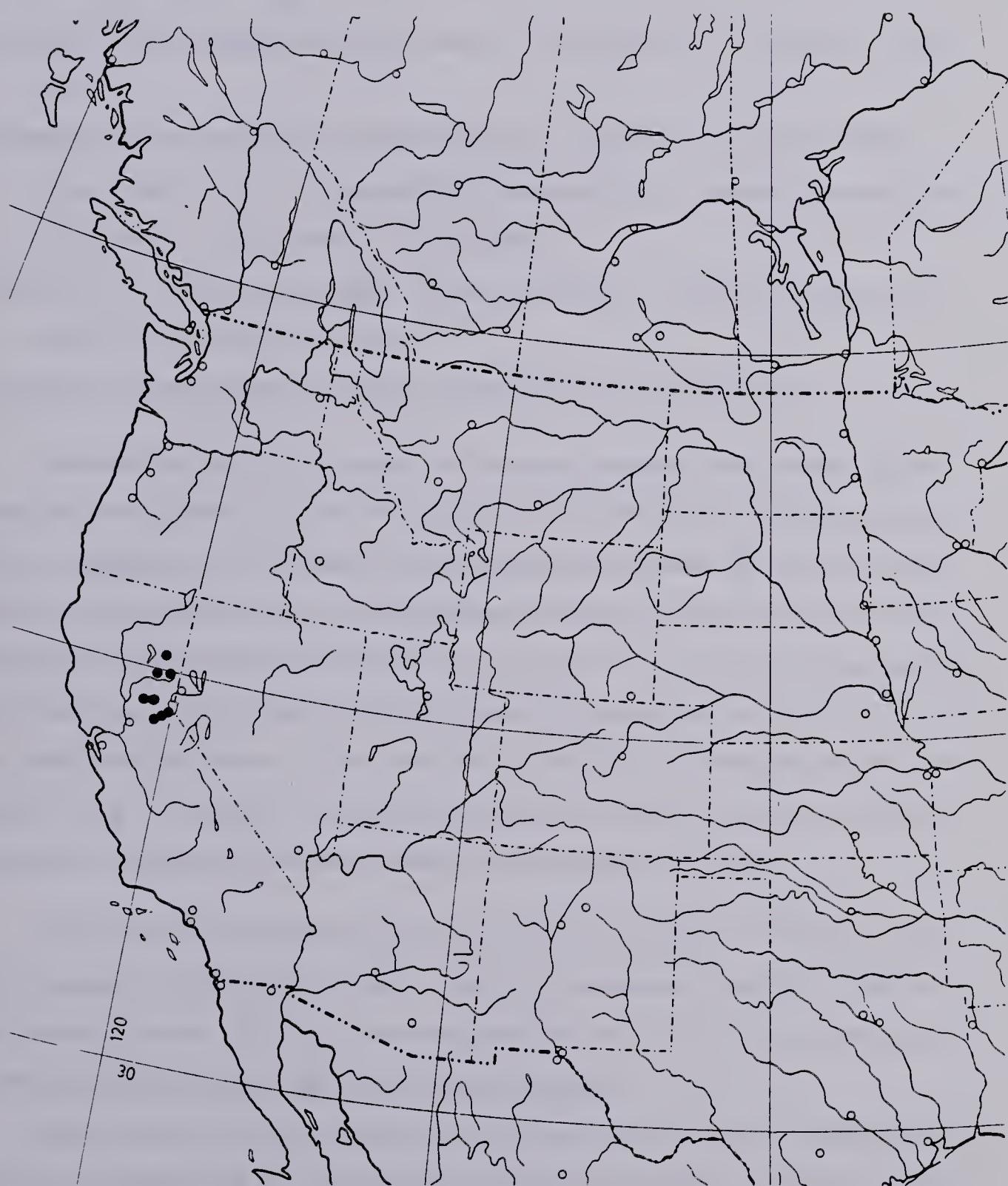
Plant densely woolly tomentose throughout; leaves 15 to 50 mm long, 9 to 25 mm wide, ovate to elliptic, margins entire; chasmogamous flowers small, lower petal about 9 mm long; cleistogamous flowers unknown;  $2N = 12$ .

In terms of leaf flavonoids, *tomentosa* contained kaempferol, apigenin, and quercetin as had *nuttallii*. This was intermediate between *major*, and *praemorsa* and *bakeri*. *V. tomentosa* appears to be rare and restricted to the Sierra Nevada of California at elevations of 1500 to 2000 m (Figure 18). It has been found in dry gravelly soil of open coniferous forests. *V. tomentosa* has an extended flowering season from June to August.

*V. tomentosa* was without a doubt distinctive both in terms of its morphology and its diploid chromosome complement.



Figure 18. Distribution of *Viola tomentosa* Baker & Clausen.





*Viola bakeri* Greene. Pittonia 3: 307. 1898.

Type: Bear Valley mountains, Siskiyou County, California. June, 1896. UC (holotype), DS! (isotype).

*Viola bakeri* Greene subspecies *grandis* Baker. Madroño 10: 117. 1949d. (Nom. Nud.)

*Viola bakeri* Greene subspecies *shastensis* Baker. Madroño 15: 203-204. 1960.

Type: Baker 13045. "Postpile Camp, altitude 6000 ft., western Tehama County, California." July 1, 1955. UC! (holotype).

*Viola nuttallii* A. Nelson variety *bakeri* (Greene) Hitchcock. Vascular plants of the Pacific Northwest 3: 447. 1961.

*Viola bakeri* Greene subspecies *puberulenta* Baker (mss., not published).

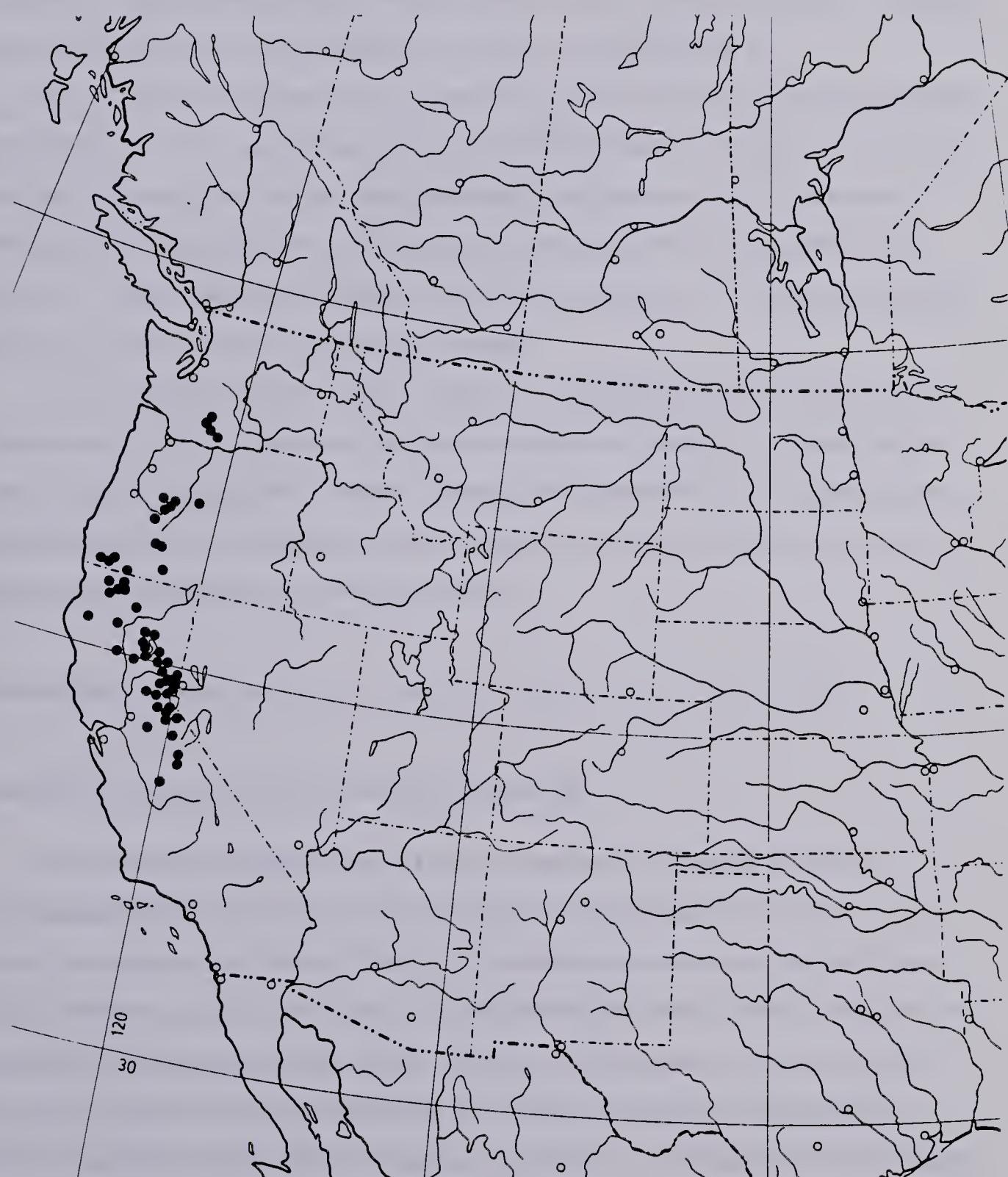
Subcaulecent early in the season and displaying elongated stems in later growth; radical and caudine leaves 20 to 40 mm long and 10 to 15 mm wide, ovate-lanceolate to ovate or elliptical, 2.1 to 2.7 times as long as wide, bases cuneate (55 to 80°), margins always entire and ciliate; more or less pubescent throughout, hairs 0.3 to 0.4 mm long, occasionally hirsute; lower chasmogamous flower petal 10 to 15 mm long; style capitate and retroseely bearded on sides as in all Nuttallianae; cleistogamous flowers 3.5 to 8.0 mm long; seeds dark red-brown, 1.8 mm wide and 3.1 mm long, 1.7 times longer than wide, weight 4.2 mg; caruncle 29% the length of the seed but not extending beyond the tip of the seed and covering the funiculus; pollen 44 um in diameter;  $2N = 48$ .

*Viola bakeri* was distributed from the Cascades of southern Washington to the Sierra Nevada of California (Figure 19). It occurs at elevations from 900 to 3800 m, most commonly above 1700 m. Flowering time peaked from mid June to mid July, but sometimes occurred as early as May and as late as August.

*Bakeri* may be easily distinguished from *tomentosa* by the shorted length and lower density of its pubescence, by its chromosome number and by its more diverse flavonoid profile. Leaves of *bakeri* contained luteolin derivatives, as well as apigenin, kaempferol and quercetin derivatives.



Figure 19. Distribution of *Viola bakeri* Greene.





*Viola bakeri* may be distinguished from *nuttallii* primarily by its entire leaf margins and by its distribution east of the Rocky Mountains. The true distinction would be chromosome number, *bakeri* is an octoploid and *nuttallii* is a tetraploid. The characters of ovary pubescence and margin ciliation were not always consistent within the taxa and were, therefore, not applicable in distinguishing them (Table 8).

Baker (1949c) mentioned two subspecies *typica* and *grandis* without descriptions or explanations. The invalid subspecies *typica* should properly have been referred to as *bakeri*, priority being given to the oldest published applicable epithet. Subspecies *grandis* was not validly published in receiving no Latin description or designated type specimen. Baker (1960) maintained *grandis* as "nomen nudum". He deferred properly describing it due to a lack of continuity in its range.

The subspecies *shastensis* (Baker, 1960) was described as differing from *bakeri* by the presence of short appressed hairs on the sepals and capsules. These features (Table 8), were not consistently reliable in distinguishing between taxa. Creating a new subspecies based on inconsistent character states could not be justified by this study, and the name was considered synonymous with *bakeri*.

***Viola praemorsa* Dougl. ex Lindl.** Edward's Bot. Reg. 15: pl. 1254. 1829.

For synonymy and typification see subspecies headings.

An herbaceous perennial from a short, occasionally praemorse rootstock; appearing acaulescent but stem elongating during the growing season; 6 to 20 cm high; glabrous or conspicuously hirsute throughout; radical leaves various but mostly broadly ovate to obovate; caudine leaves ovate to oblong-lanceolate, base cuneate to truncate or subcordate; margins sub-entire to serrate; corolla of chasmogamous flowers yellow, lower petal up to 20 mm long including the spur; style 2.5 mm long, capitate head retroseely bearded on sides; capsule glabrous to puberulent; cleistogamous flower sepals 3.5 to 8.3 mm long; seeds of various colours from medium to dark brown; mostly 1.6 times longer than wide; caruncle short, globose, not extending beyond the seed apex or covering the funiculus; pollen diameter 41 to 45  $\mu\text{m}$ ;  $2N = 36, 48$ .



*V. praemorsa* occurs in the western half of the Nuttallianae range from southwestern Alberta and the Intermountain regions of Utah west through the Great Basin to Vancouver Island and central California.

*Viola praemorsa* is broadly defined here, in the tradition of Baker (1957), based on morphological variations in leaf shape, pubescence, and seed morphology. *V. praemorsa* specimens can be easily recognized by their hirsute pubescence, if present, larger leaf size, and the distinct caruncle form of the seeds. A wide array of structural variation exists in this species throughout its range. The three subspecies recognized in this study exhibited gradual intergradation whose intermediates are difficult to identify and classify. The influences of physical and environmental factors, along with hybridization and introgressive influences contribute to the multifarious nature of the structural forms observed. These subspecies represent variants defined primarily on extremes in leaf form, whose character states tend to overlap. The relationships of these taxa are discussed in more detail later.

#### Key to the subspecies of *Viola praemorsa*

1. a. Cauline leaves mostly short, 23 to 40 mm long; ovate to broadly ovate, length to width ratio of 1.6; blade base approaching truncate (106 to 172°), margins serrate; seeds dark brown, widely ovate, length to width ratio 1.4, 5.8 mg each, caruncle relatively short, 0.7 mm long;  $2N = 36$  . . . . . subspecies *praemorsa*
1. b. Cauline leaves mostly longer, 33 to 80 mm; ovate to elliptical, length to width ratio of 1.8 to 3.2; basal angle 63 to 112°, margins subserrate to serrate; seeds medium brown, ovate, 1.5 to 1.7 times longer than wide, 3.9 to 5.1 mg; caruncle 0.8 to 1.0 mm long;  $2N = 36, 48$ .
2. a. Cauline leaves 33 to 62 mm long, 15 to 23 mm wide, ovate to broadly ovate;  $2N = 36, 48$  . . . . . subspecies *linguaefolia*
2. b. Cauline leaves large, 60 to 80 mm long, 23 to 31 mm wide, elliptic;  $2N = 36, 48$  . . . . . subspecies *flavovirens*



*Viola praemorsa* Dougl. ex Lindl. subspecies *praemorsa*

*Viola praemorsa* Dougl. ex Lindl. Edward's Botanical Register 15: plate 1254. 1829.

Type: "According to Mr. Douglas, in dry upland soils, under the shade of solitary Pine trees on the banks of the Columbia, and the plains above the river Aguilar, in California. Our drawing was made in the Garden of the Horticultural Society in 1828." CGE (holotype), DS! (photograph of holotype).

Lectotype: M. S. Baker 9634, South Tacoma, Washington. UC! (lectotype); WS!, RM!, MO!, DAO! (isolectotypes). Lectotypification redundant.

*Viola nuttallii* variety *praemorsa* (Dougl. ex Lindl.) S. Wats. *Botany, Clarence King Expedition; U.S. Geological Exploration of the Fortieth Parallel* 5: 35. 1871.

*Viola nuttallii* *praemorsa* (Dougl. ex Lindl.) Piper. Contr. U.S. Nat. Herb. 11: 393. 1906.

A herbaceous perennial, with short leaves, 20 to 40 mm long, length to width ratio of 1.6; leaf bases approaching truncate; pubescence densely hirsute at least on petioles and young leaves; seeds widely ovate, length to width ratio only 1.4, and weighing 5.8 mg, caruncle relatively short, 0.7 mm long, seed coat dark brown;  $2N = 36$ .

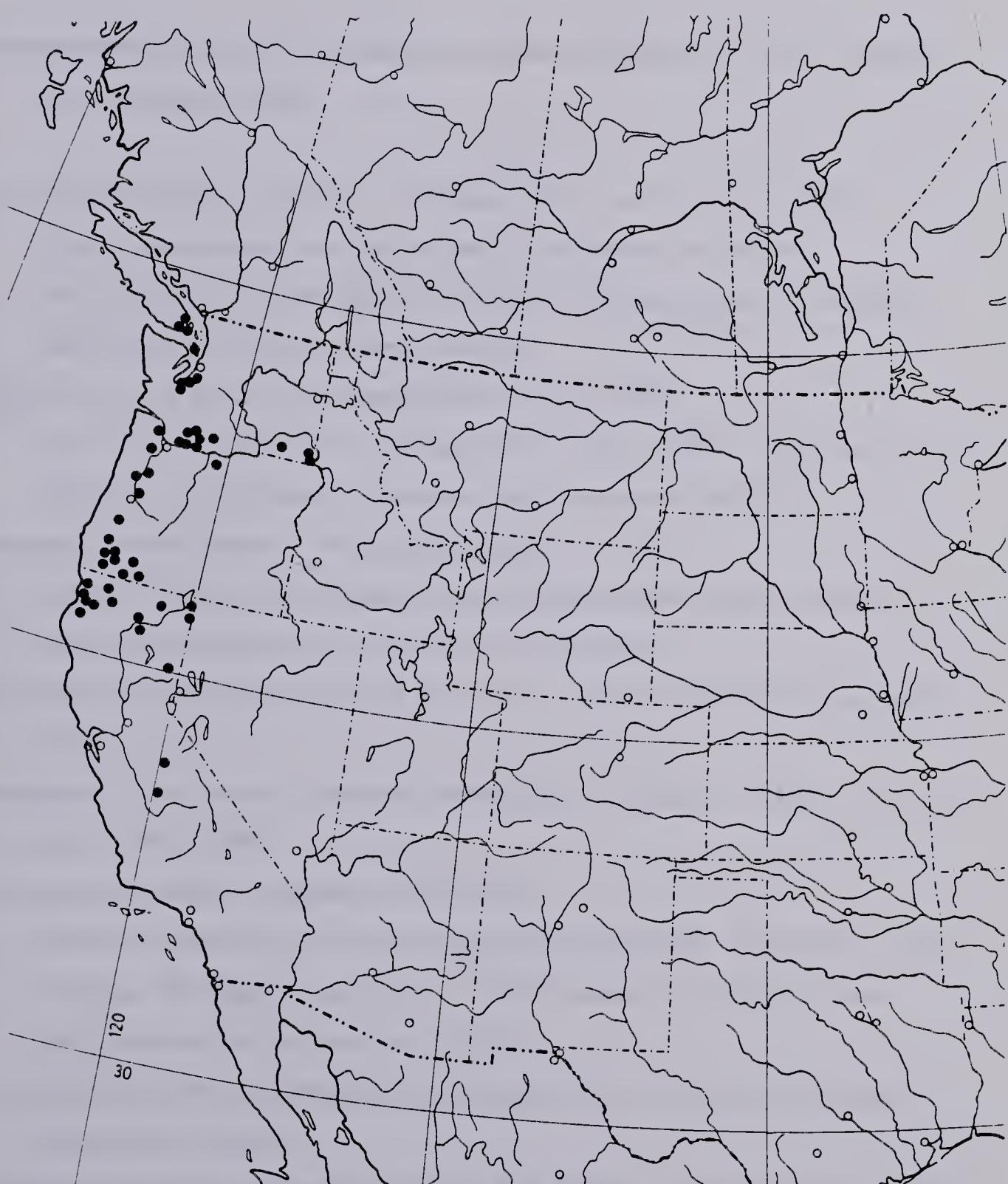
This taxon occurs from the Saanich Peninsula of Vancouver Island south along the Coastal Ranges to northern California (Figure 20). Invaginations inland were evident along the Columbia River in Washington and toward the Sierra Nevada in California.

Subspecies *praemorsa* was commonly found at elevations of 100 to 2500 m, though a few inland collections were found at elevations up to 7000 m. Flowering times were generally middle of April to early May but have been observed from March to June.

This taxon was first collected by D. Douglas near the mouth of the Columbia River. The published drawing was made from transplanted specimens at the Botanic Garden, but the original specimens appeared to have been lost. Most workers accepted the drawing in lieu of a holotype (Brainerd, 1921). From a photograph (DS 558332), it appears that a collection purchased from Lindley's herbarium had found its way to Cambridge and may well be the original type specimen. The label reads: "North West America, Douglas."



Figure 20. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies  
*praemorsa*.





"Cambridge Botanical Museum. Herb. J. Lindley, PhD. Purchased 1866." Baker (1949c) lectotypified this taxon with a collection from Tacoma, Washington which is considered to be redundant.

*Viola praemorsa* Dougl. ex Lindl. subspecies *linguaefolia* (Nutt. ex T. & G.) Baker. *Brittonia* 9: 226. 1957.

*Viola linguaefolia* Nutt. ex T. & G. *A Flora of North America* 1: 141. 1838.

Type: "Kamas prairie, near the sources of the Oregon, Mr. Wyeth."

PH! (holotype); GH! (photograph of holotype), WS! (photograph of holotype); BM! (isotype); PH! (photograph of isotype),

*Viola erectifolia* A. Nels. *Botanical Gazette* 29: 143. 1900.

Type: *A. Nelson 5481*, Henry's Lake, Idaho, June 22, 1899. RM! (holotype); PH!, MO!, RM!, UCI!, US!, and GH! (isotypes); ALTA (topotype, DF 622).

*Viola gomphopetala* Greene. *Plantae Bakerianae* 3: 11. 1901.

Type: *C. F. Baker 225*, on open hillsides of Grand Mesa [Colorado], June 23, 1902. RM! (holotype); RM!, WS!, UCI!, GH! (isotypes).

*Viola nuttallii linguaefolia* (Nutt. ex T. & G.) Piper. *Flora of Southeast Washington*. 1914.

*Viola nuttallii* Pursh variety *linguaefolia* (Nutt. ex T. & G.) Jeps. *A Man. Fl. Plants of Calif.*, 645. 1925.

*Viola xyloorrhiza* Suks. *Werdenda* 1: 25. 1927.

Type: *W. Suksdorf 10200*, east of Husum, Klickitat County, Washington, 10 May, 30 June, 1919 and 1 June, 1920. WS! (holotype); UCI!, MO!, CAS!, and DAO! (isotypes); ALTA (topotype, DF 397).

*Viola praemorsa* variety *oregana* (Baker and Clausen) Peck. *Man. High. Plants of Oregon*, 486. 1941.

*Viola praemorsa* variety *linguaefolia* (Nutt. ex T. & G.) Peck. *Madrono* 6: 135. 1941.

*Viola praemorsa* Dougl. ex Lindl. subspecies *arida* Baker. *Brittonia* 9: 227-228. 1957.

Type: *M. S. Baker 11462* in fruit, outskirts Klamath Falls, Oregon. UCI! (holotype); RM!, DS!, MO!, and DAO! (isotypes).



Paratype: M. S. Baker 12086 in flower, from same colony. UC! (holotype); MO!, RM!, DAO! (isotypes); GH! (photograph of holotype).

*Viola praemorsa* Dougl. ex Lindl. subspecies *oregona* Baker. Brittonia 9: 228-229. 1957.

Type: *M. S. Baker* 8862. "The type locality is about half mile west of Klamath River Bridge on the Klamath Falls to Medford hwy c. 20 miles from Klamath Falls." DS! (holotype); RM!, MO!, and DAO! (isotypes); GH! (photograph of holotype), ALTA (topotype, DF 443).

Leaves tongue-shaped, 33 to 62 mm long and 15 to 23 mm wide, ovate to narrowly ovate, blades cuneate (63 to 112°), margins subserrulate to serrulate; seeds brown to red-brown, 1.5 times longer than wide, weighing up to 5.1 mg with caruncles less than 1 mm in length; pollen large, 37 to 45 µm in diameter; 2N = 36, 48.

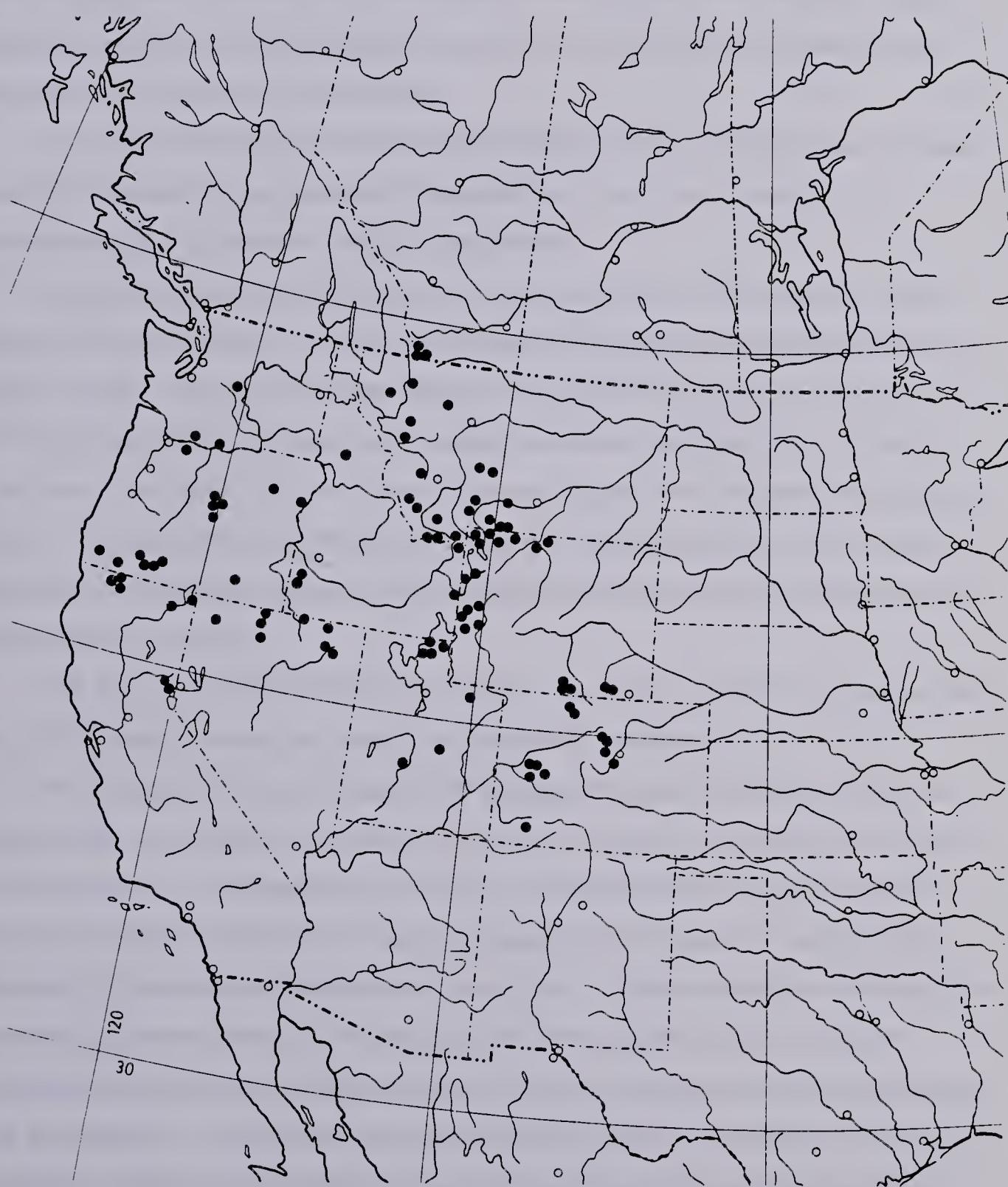
Occurs from the Rocky Mountains of southwestern Alberta through Wyoming, Colorado and Utah, through the Intermountain region into the Great Basin ranges in northern Nevada and California, Oregon and Washington (Figure 21) at elevations of 1300 to 3500 meters above sea level. The peak flowering times were from mid-June to early August, but some flowers appeared as early as the first week of May. This distribution range overlapped significantly with *bakeri* in central Washington, Oregon, and California; with subspecies *praemorsa* along the Columbia River and in the Sierra Nevada; with subspecies *f/avovirens* in Idaho, Washington and Wyoming; and, with *tomentosa* in the Sierra Nevada. The many morphological variants which were observed in this study may be symptomatic of these sympatric distributions which lead to the possibilities of hybridization and / or introgression.

The confusion in this taxon arises from the range of variation which exists in leaf shape and pubescence, and the presence of two different chromosome complements. The confusion in the relationships of the subspecies of *praemorsa* can be illustrated by the number of combinations which have been published by various authors.

A specimen at PH was annotated as the type specimen of *Viola linguaefolia*. Its label read "V. nuttallii / linguaefolia / HK... \*Hookeri. Kamas Prairie near the source of the Columbia (Nutt. from Wyeth)." This label matches the information given in



Figure 21. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies  
*linguaefolia* (Nutt. ex T. & G.) Baker.





the publication (Nutt. ex T. & G.). The handwritting on this specimen also matches that of a specimen located in the British Museum (photographs!) dated June 20. The BM specimen was surely an isotype of the original collection on the Columbia, formerly known as the Oregon River. Nuttall was in Philadelphia from 1836 to 1841 (Brainerd, 1921), during the time when Torrey and Gray published their *Flora* (1838), and it seems most likely that the PH specimen is the holotype.

As shown in the numerical analysis *erectifolia* A. Nels. and *gomphopetala* Greene were both consistently associated with *linguaefolia*. Both were collected in mountainous regions within the range of *linguaefolia*.

Another source of confusion arises from the discovered misapplication of the epithet "major" to this taxon. Piper (1906) listed four specimens as representative of *V. nuttallii major*. Three of these specimens were examined and found to be referable here rather than with *major sensu* Hook. in both appearance and location. In listing *major* here, a confusion as to its correct application began which has been perpetuated to this day. It was apparent that Piper did not see the original specimen; if he had seen the specimen there would have been no need to cite Hooker's authority for the synonymy of *glareosa* Dougl. and *major*.

The description and distribution of Jepson's combination (1925) matched perfectly that of *linguaefolia*, though he cited no representative specimens.

It was almost one hundred years after Douglas published *praemorsa* (1829) that workers began to recognize *linguaefolia* as being more closely affiliated with *praemorsa* than with *nuttallii*. Morton Peck (1941) was the first to combine *linguaefolia* and *major* as varieties of *praemorsa*, though no explanations were given. Baker (1949c) listed both of these taxa as subspecies of *praemorsa*. It was not apparent whether type specimens had been examined, but detailed descriptions, distributions, and lists of representative specimens included at the time of publication permitted these combinations to be equated here. Baker offered several methods by which to distinguish *major* and *linguaefolia* as he understood them, including leaf and capsule pubescence, leaf width and basal angle, and chromosome complements of  $2N = 48$  and 36, respectively. As already mentioned, relative puberulence of capsules and density of leaf pubescence have been shown to be inconsistent (Table 8), and therefore, unreliable as distinguishing



characters. The extremes of leaf shape used to define these two conceptual subspecies have been shown to represent the ends of a continuum between which no lines could be drawn based on present knowledge.

It is curious to mention at this point that Peck (1941) had published a new combination of *oregana*, apparently a spelling mistake, before the subspecies was actually described (Baker, 1957). Since no explanation was given, one can only assume that he had prior communication with Baker and Clausen before the 1957 publication.

Baker's subspecies *oregona* and *arida* (1957) were shown, in the numerical analysis (Figure 13, Appendix 4), to be morphologically indistinguishable within the continuum of attributes representing subspecies */inguaefolia*. Subspecies *arida* was distinguished because it was octoploid and had puberulent cleistogamous capsules in comparison to the glabrate ones in *major sensu* Baker and *praemorsa*. Subspecies *arida* had been found in a few scattered locations in eastern and southern Oregon and northern California. This range overlaps those of */inguaefolia* and *praemorsa*. I agree with Clausen (1964) who believed that the creation of a new subspecies based on tenuous characters within the range of a taxon from which it could not be reliably distinguished was unjustifiable.

Subspecies *oregona* is restricted to four sites in southern Oregon. It is distinguished by shorter than normal pubescence for the *praemorsa* group, with a leaf shape approaching that of *bakeri* but with serrate margins. The numerical analysis included this taxon morphologically with */inguaefolia* but chemically as intermediate between */inguaefolia* and *bakeri* (Appendix 4). This evidence supports the suggestion of possible hybridization between the two (Baker, 1957) due to their cytological, morphological and flavonoid similarities in conjunction with their overlapping distributions.

The broadly defined subspecies */inguaefolia* was observed to display at least two different flavonoid patterns. In one group, 12 to 20 different flavones and flavonols (apigenin, luteolin, kaempferol, and quercetin derivatives) were present in each specimen extracted. The types of flavonoids and their patterns resembled those found in *bakeri* and *flavovirens*. The second group was found to contain only flavonols, derivatives of quercetin and kaempferol. The profiles of these variants represents a decrease in the diversity of flavonoids to only seven compounds in total. No correlation with



morphology, cytology or geographic distribution could be made for these two chemical variants except that all of the reduced profile specimens had a chromosome count of 48.

***Viola praemorsa* Dougl. ex Lindl. subspecies *flavovirens* (Pollard) Fabijan stat. et comb. nov.**

*Viola flavovirens* Pollard. Bull. Torrey Bot. Club 24: 405. 1897.

Type: A. A. Heller 3156 "at Lake Waha, Nez Perces Co., Idaho, June 3 and 4, 1896". PH! (holotype); DAO!, MO!, WS!, UC!, and US! (isotypes).

Leaves 60 to 80 mm long and 23 to 21 mm wide, when fully matured, margins regularly serrate-crenate, pubescence generally over 0.5 mm long, seeds about 4.1 mg, caruncle less than 30% of its length; pollen 34 to 37  $\mu$ m in diameter;  $2N = 36, 48$ .

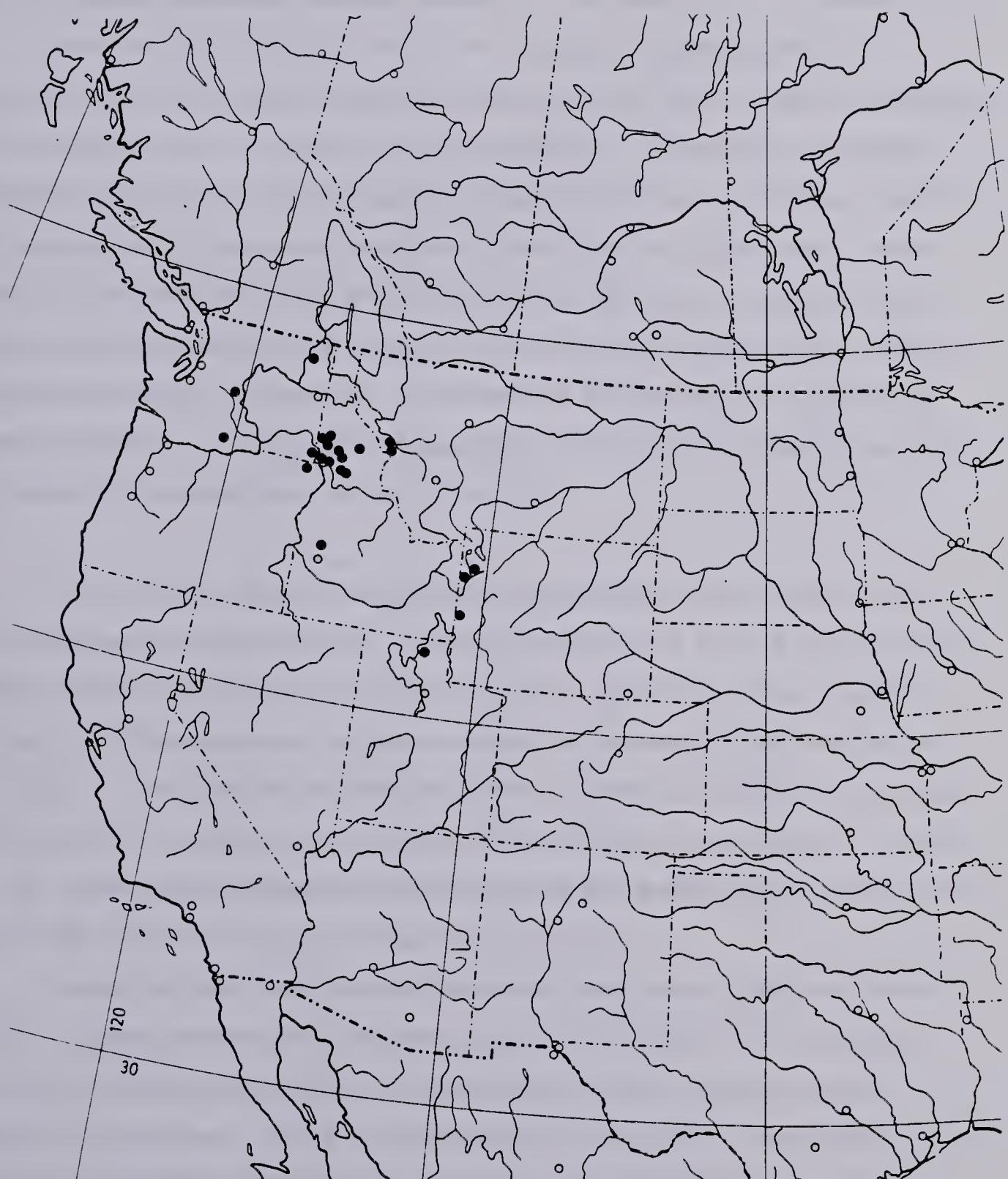
Occurs in the Rocky Mountains of the Idaho panhandle and adjacent Washington and Montana, with a few distant populations in western Wyoming and northern Utah (Figure 22). Found at lower elevations of 900 to 1200 m. The flowering period was from late April to early or mid June.

The collection number of the type specimens appears to have been misprinted at the time of publication as 3106 instead of the 3156 indicated on the type specimen which was from the correct location and on the correct date. The specimen at UC of the same collection without a collection number must be referred to here as well since it was collected at the same time and place.

Beginning with Piper and Beattie (1914), *f/avovirens* has been treated as synonymous with *V. praemorsa* subspecies *major* by all authors. Annotations on herbarium sheets by M. S. Baker and J. Clausen indicated they may have considered reassigning it to variety or subspecies level. No such combination was ever published.



Figure 22. Distribution of *Viola praemorsa* Dougl. ex Lindl. subspecies  
*flavovirens* (Poll.) Fabijan.





#### IV. DISCUSSION

This investigation of the *Viola nuttallii* complex resulted in the recognition of eight taxa (five species and three subspecies) based on their differences in ploidy level, morphological attributes and flavonoid chemistry: *V. vallicola*,  $2N = 12$ ; *V. nuttallii*,  $2N = 24$ ; *V. praemorsa*,  $2N = 36, 48$ ; *V. bakeri*,  $2N = 48$ ; and *V. tomentosa*,  $2N = 12$ .

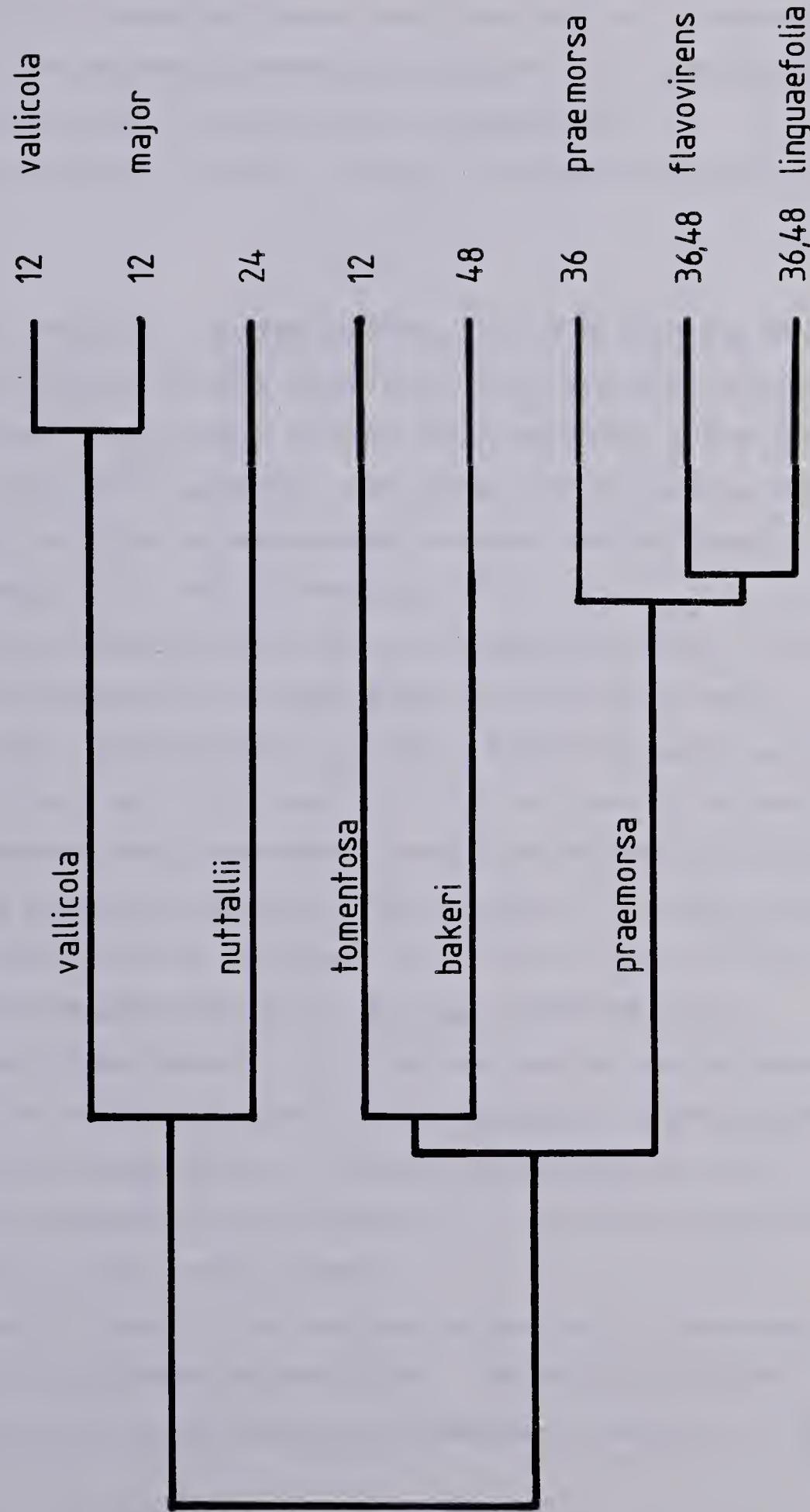
Departures from the most recent, detailed analysis (Clausen, 1964) include the recognition of two subspecies of *V. vallicola*: *vallicola* and *major*; *V. praemorsa* is envisaged as embracing a wide range of morphological and chemical diversity the extremes of which are recognized as the subspecies *praemorsa*, *flavovirens*, and *linguaefolia*. In the absence of morphological or geographical differences between *V. linguaefolia* and *V. praemorsa* subspecies *major* and *oregona*, taxa recognized by Clausen (1964), these are included in subspecies *linguaefolia*. This treatment is supported by cytological and chemical evidence. A diagrammatic representation of the taxonomic relationships among the members of the Nuttallianae is given in (Figure 23).

The *V. nuttallii* complex has long been recognized as a polyploid series with a basic chromosome number of  $x = 6$ . Chromosome counts of  $2N = 12, 24, 36$  and  $48$  obtained in this study confirm all previous counts reported for this complex (Table 5). It is known that in related species, cell sizes increase with increased ploidy level (Sax and Sax, 1937). This principle was confirmed for the *V. nuttallii* complex, in which pollen grain diameter is correlated with an increase in chromosome number (Figure 2). Pollen size can, therefore be employed in determining ploidy level in this complex, and may be of considerable utility in any future investigation of these taxa.

Hybrids had been reported in the literature by both Clausen (1964) and Davidse (1976). These included hybrids between subspecies *linguaefolia* and *V. utahensis* (Purpureae) which produced an  $n = 18$  individual; and between *linguaefolia* and *V. douglasii* (Chrysanthae). This latter hybridization was believed by Clausen (1964) to have given rise to what Baker (1949c) considered to be *V. praemorsa* subspecies *arida*. Hybridizations between *V. tomentosa* and various members of the Purpureae and Chrysanthae subsections have also been found (Table 2). The presence of these reported hybrids indicates a close relationship between the *praemorsa*, *tomentosa*



Figure 23. Taxonomic relationship of the members of the *Viola nuttallii* complex.





species and other subsections in the Chamaemelanium.

A new hybrid (Table 5) was discovered between subspecies */inguaeolia* ( $2N = 36$ ) and *V. purpurea* ( $2N = 12$ ), subsection Purpurea, which grew intermixed at a location in California. The hybrid had a mitotic chromosome complement of  $2N = 28$  and pollen viability was estimated at only 7%, indicating that it was probably sterile.

No hybrids involving *V. vallicola* or *V. nuttallii* were observed nor have any been reported.

Prior to this investigation, very little was known about the flavonoid chemistry of *Viola*. The genus had been reported to contain the following flavones and flavonols: quercetin, kaempferol, luteolin, apigenin, violanthin, vitexin, saponarin, orientin, and isoorientin (Bate-Smith, 1962; Wagner *et al.*, 1972; Gibbs, 1974; and Harborne, 1975). The only taxonomic work within the genus involving flavonoids, used only flavonoid profile analysis (Stebbins *et al.*, 1963 and McPherson, 1972).

The present study identified luteolin aglycone, oxygenated glycosides of apigenin, luteolin, kaempferol, and quercetin; a 6-methyl apigenin glycoside; and, a 6-methyl kaempferol glycoside. The most common glycosides were rutinose, glucose, galactose, rhamnose and arabinose (Figure 11 and Table 6). Further identification of the unknown compounds is expected to reveal the presence of some of the other previously reported flavonoids. Their presence is suspected from the comparison of Rf values with those reported in the literature (Harborne, 1967; Mabry *et al.*, 1970; and Jay *et al.*, 1975).

Despite the incomplete identification of all extracted flavonoids, careful examination of flavonoid spot patterns on two-dimensional paper chromatography and comparison of the major types of aglycones proved to be helpful in evaluating taxonomic boundaries and relationships among taxa. Flavonoid profiles have proven useful in related work in *Viola* subsection Purpurea (Stebbins *et al.*, 1963) where the ancestry of amphiploids and introgressants were investigated.

In the *V. nuttallii* complex, it was possible to correlate flavonoid pattern with ploidy level, morphological features, and distributions. The phytogeographic and phylogenetic implications of flavonoid analysis will be discussed for each taxon. The



proposals for the evolutionary history of the members of the Nuttallianae are summarized in Figure 24.

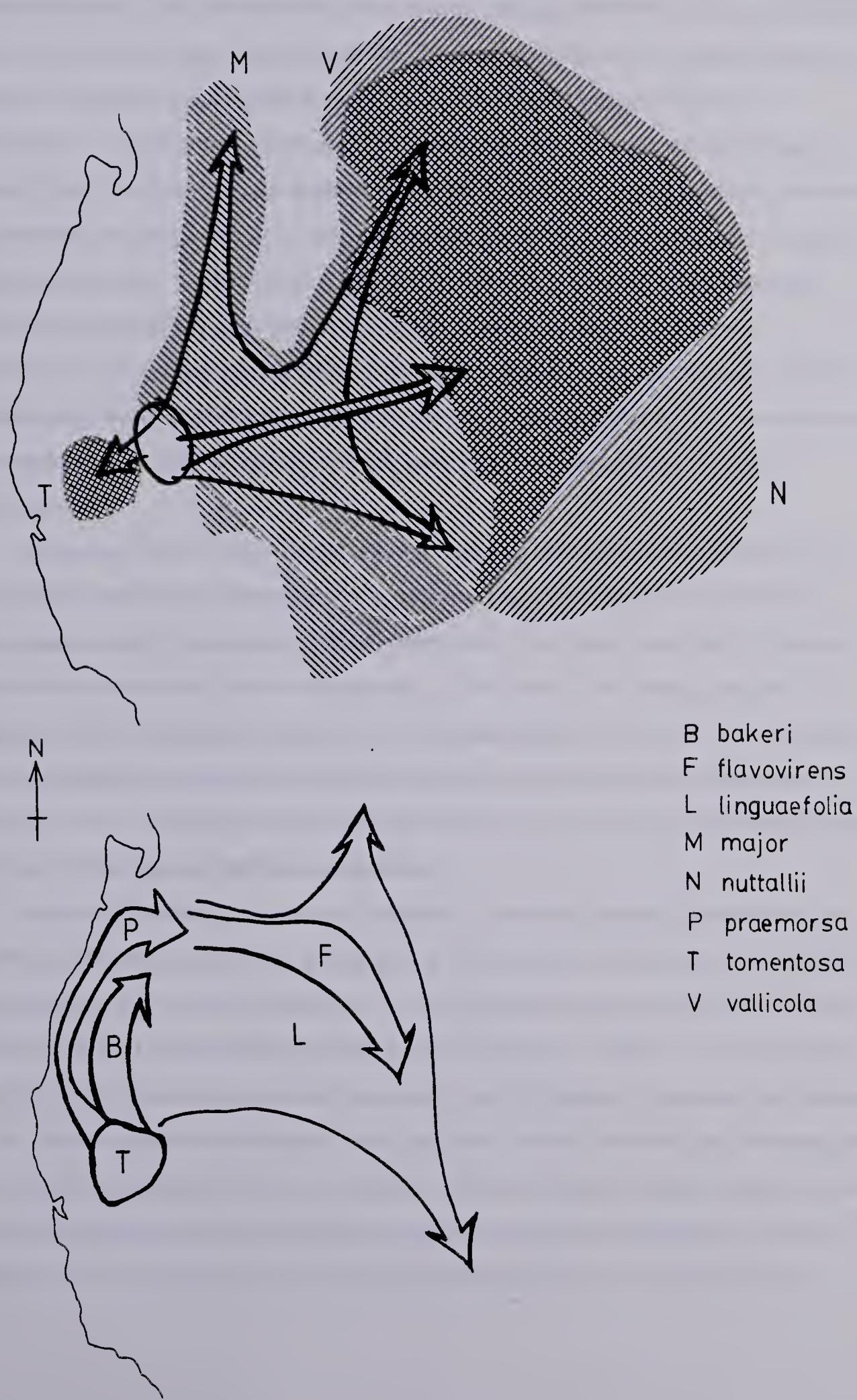
The chromosome number of  $2N = 12$  for *V. vallicola* for 30 populations from British Columbia, Alberta and Saskatchewan (Table 5) supplements 19 previous counts (Davidse, 1976) from Idaho, Nevada, and Utah. Together, these reports cover a large portion of the range of this taxon. Only the easternmost populations in the range remain to be examined.

*Viola vallicola* specimens contained a total of eleven different flavonoid compounds: attributes 1, 2, 3, 4, 5, 6, 7, 9, 10, 11 and 29 (Table 7). Examination of chromatographic profiles revealed the presence of two distinct flavonoid races in this species plus a third pattern which appeared to be intermediate. None of these patterns could be correlated with any morphological feature examined in this study (Appendix 3), they could, however, be correlated with geographic occurrence (Figure 16). Subspecies *vallicola* contained primarily (frequency of 50% or greater) apigenin derivatives , compounds 2 and 3 (Table 7), and was found in Alberta and Saskatchewan. Subspecies *major* was found to contain predominantly kaempferol glycosides, compounds 1 and 9, and occurred in the interior of British Columbia and Oregon. A number of specimens from the Great Basin regions of Idaho and Nevada were found to be intermediate; they lacked compounds 2 and 9, but possessed compounds 1 and 3, and also contained two novel compounds, 5 and 10.

The recent elucidation of the general biosynthetic pathways of flavonoids in a number of higher plant taxa (Giannasi, 1978) indicate that the flavone apigenin represents one biosynthetic step from the precursor flavanone while kaempferol represents two biosynthetic steps: from flavanone to flavanonol to flavonol. Subspecies *major*, which possessed flavonols, may be considered further removed from the ancestor than subspecies *vallicola* because of its possession of these more complex flavonoid characters. The intermediates could be considered as either ancestral to one, or both subspecies, or as introgressive hybrids between two ancestral chemical forms. It would be impossible to prove which of these possibilities was true until specimens from the entire range of *V. vallicola* were examined for their flavonoid profiles and all compounds



Figure 24. A proposal for the evolutionary history of the *Viola nuttallii* complex.





were positively identified.

The Great Basin region, lying in the area of continuous distribution across the continental divide, could represent the point where one subspecies migrated across giving rise to the other; the area of dispersal of the species resulting in two chemical variants; or the area of overlap of two ancestral chemical forms with the opportunity for introgression. The hypothesis of genetic flow across this region is supported by the apparent filtering of biosynthetic capabilities across it (Table 7). Compound 2, found at 96% frequency in subspecies *vall/ico/a*, was present in 17% of the intermediates and 0% of subspecies *major*. Compound 1, present in 100% of the *major* specimens and intermediate specimens, was found in one specimen of subspecies *vall/ico/a*. Compound 10 was present in only 50% of the intermediates, and was present in 17% of the subspecies *major* specimens. The only unique compound present in the intermediate was compound 5. Until all compounds are identified, this interpretation remains speculative.

Alston and Turner (1962) used flavonoid patterns to investigate introgression in natural hybrids and concluded that progeny resulting from introgressive hybridization rarely contain unique compounds. More recent work (Levy and Levin, 1971) indicates that novel compounds are produced in hybrids. The novel compounds were the probable result of repression or suppression of glycosylation in the hybrid, which would result in less highly glycosylated flavonoids than those found in either of the parents. The situation for *V. vall/ico/a* could be clarified simply by examining the flavonoids present in artificial hybrids between the two subspecies.

Three phylogenetic scenarios are proposed. With the gradual, continuous uplift of the Rocky Mountains during the Quaternary, *V. vall/ico/a* or its ancestral form became split into eastern and western populations. The Oligocene cooling trend also resulted in gradual migration of vegetation to lowland areas (Tidwell *et al.*, 1972). The three major glaciations of the Pleistocene covered the eastern Sierra Nevadas, Cascades, northeastern Utah, the Uinta Mountains, and parts of the Great Basin, as well as the Rocky Mountains and most of western Canada (Tidwell *et al.*, 1972). The Intermountain region however, was semiarid during these maximum advances perhaps allowing for the meeting of the two subspecies. The consequence was introgressive hybridization with disruption of



flavonoid biosynthesis at the genetic or regulatory levels (Levy and Levin, 1971)

If before the Pleistocene glaciations *V. vallicola* or its ancestor was found on only one side of the Rocky Mountains, migration south to the Great Basin area during glacial maxima, may have afforded it an opportunity to migrate across the continental divide and expand its range concomitant with an evolutionary change in flavonoid biosynthesis.

The third proposal, favored by the author, suggests that isolation during post-glacial migration northward on either side of the mountains could account for the differentiation of two subspecies. The loss of flavonoid synthesizing capability for flavonols in subspecies *vallicola* is incomplete because some specimens appeared to accumulate kaempferol and quercetin derivatives. Genetic isolation, therefore, cannot be absolute even in the northern most portion of the range. Further flavonoid surveys would help to establish whether the subspecies were a purely northern phenomenon or represent an east-west differentiation.

Twelve populations of *V. nuttallii* from Alberta and Saskatchewan were found to possess somatic chromosome counts of  $2N = 24$  (Table 5), confirming the two previous counts for this taxon (Baker, 1949c; Clausen, 1964). One of these counts was from a transplant of nursery stock from Colorado; the other from buds fixed by W. C. McCalla (7355, ALTA) from a population in Calgary, Alberta.

*Viola nuttallii* contains one flavone (3, an apigenin derivative), several flavonols (1 and 4, kaempferol 3-O diglycosides; and 6, 7, 8, quercetin 3-O and 3,7-O diglycosides) and other flavonoids (8, 10, 12, at low frequencies; see Table 7). Morphologically, *V. nuttallii* is most similar to *V. vallicola*, differing principally in leaf shape, and seed size and colour (Figure 12, Appendices 2 and 3). Chemically, *nuttallii* is most similar to *V. vallicola* subspecies *vallicola*, with 56% of their flavonoids in common and *V. tomentosa*, with 50% of their flavonoids common (Table 7) and which it approaches in leaf basal angle and seed colour.

*V. vallicola* and *V. nuttallii* are sympatric east of the Rocky Mountains (Figures 14 and 16) and some populations in Alberta and Saskatchewan were observed to grow in close proximity. No hybrids were observed and none have been reported in the literature.



*V. nuttallii* was thought to be an autotetraploid of *V. vallicola* by Baker (1957), while Clausen (1964) speculated that it might have originated from an as yet undiscovered diploid race of *V. nuttallii*. No such diploid has been discovered. Morphological and chemical evidence presented in this study suggest the possibility that *V. nuttallii* is an allotetraploid of *V. vallicola* and *V. tomentosa* (Table 7).

Levy (1976) found that colchicine-induced autotetraploids of *Phlox* exhibited novel glycoflavonoids, loss of diploid glycoflavonoids and deregulation of glycoflavonoid biosynthesis. All of these features can be observed in the differences in leaf flavonoids between *V. nuttallii* and *V. vallicola* subspecies *vallicola*. *V. nuttallii* did not possess apigenin 7-O diglycoside (2) but did possess novel flavonoids 8 (quercetin 3-O monoglycoside), 10, and 12. Deregulation of flavonoid biosynthesis was found for compounds 1, 4, 6 and 7, both in terms of frequency and in relative intensity of spot patterns on paper chromatographs (Appendix 1).

The same comparison of lost and novel compounds, and the deregulation of flavonoid production was observed between *V. tomentosa* and *V. vallicola*. They are not sympatric at the present time, but their ranges may have overlapped at some time in the past. With the increasing Pleistocene aridity in the Great Basin and California, *nuttallii* (and *vallicola*) is proposed to have become adapted to more xeric conditions than *tomentosa*, and moved into the lowland and prairie environments (Figure 24A). *V. nuttallii* is not known west of the Rocky Mountains, its range overlaps only with *V. vallicola* subspecies *vallicola*. The ecological requirements of *V. nuttallii* are very similar to those of *V. vallicola* and may lead one to expect them to have the same distributions. However, *V. nuttallii* has a maximum elevational range of 2400 m, not as high as *V. vallicola*, and may have been unable to migrate westward over the mountains.

*Viola tomentosa*, a diploid, possessed apigenin (2 and 3), kaempferol (1) and quercetin (6, 7, 8) derivatives, and flavonoid 24 (Table 7). It had 38% of its flavonoids in common with the diploid *V. vallicola*, and 50% in common with the tetraploid *V. nuttallii*. This suggests that these taxa are related in some way. The distributions of *V. vallicola* and *V. nuttallii* do not overlap with *V. tomentosa*, which occurs in upland forest areas in the Sierra Nevada. Their ranges may have diminished in recent times due to the Pliocene



uplift of the Sierra Nevada and Cascade Ranges (Jardin and McKenzie, 1972) in conjunction with the increasing aridity and summer drought conditions on the west coast (Chaney, 1947).

A chromosome count of  $2n = 48$  (Table 5) for *V. bakeri* from Trout Lake, Washington, is the first for this taxon outside of California (Baker, 1949a). *V. bakeri* was most similar to *V. tomentosa*, whose range it overlaps in the Sierra Nevada (Figure 19). No tetraploid intermediate is known in that range, nor does there appear to be a suitable hexaploid with which it could have hybridized.

*V. bakeri* and *V. tomentosa* have only four (24%) of their flavonoids in common; three of these are quercetin derivatives (Table 7). The unique feature of *bakeri* (and *praemorsa*) specimens is the presence of a diverse array of different flavonoids and the presence of luteolin derivatives (11, 13, 15, and 17). The biosynthetic capability to produce these new flavonoids may be due to the effects of past (unknown) polyploidy or may have been introduced through hybridization with species in the closely related subsections *Purpureae* and *Chrysanthae* (Table 2).

*V. bakeri* occurs in the Sierra Nevada and Coastal ranges and occupies areas which also overlap with the subspecies of *V. praemorsa*. That *V. bakeri* possesses no compounds not found in *V. praemorsa* is a strong indication of a close phylogenetic relationship, perhaps through a common ancestor. The ancestral flavonoid pattern must then have included compounds 6, 7, 8, 11, 13, 14, 15, 16, 17, and 22, apigenin, luteolin, kaempferol and quercetin derivatives. It is also possible that hybridizations may occur between *bakeri* and octoploid */inguaefolia* and *f/avovirens* in their regions of overlap in central Washington and southern Oregon and northern California. These regions contain a number of examples of possible hybrids such as subspecies *oregona* Baker (1957) in southern Oregon. TAXMAP analysis three (Figure 13, Appendix 4) revealed that though specimens of *oregona* were morphologically clustered with */inguaefolia*, they occupied a position intermediate between *bakeri* and */inguaefolia*.

*V. praemorsa* is composed of three subspecies which represent recognizable extremes in the range of morphological variation present in the species. As outlined in



the results these variations existed in cytological, morphological, and chemical attributes. These taxa were found to contain a vast array of different flavonoids, up to 20 in one taxon, in comparison to diploid and tetraploid taxa (Table 7). One strikingly unique feature of this flavonoid profile was the presence of a new aglycone base, luteolin. The four identified luteolin derivatives (11, 13, 15, and 17) were prominent in the profiles of most specimens of *V. praemorsa* and *V. bakeri*.

Chromosome counts of  $2N = 36$  from two populations of *V. praemorsa* subspecies *praemorsa* in Washington and California, including a topolectotype (DF 414), of subspecies *praemorsa* (Table 5) confirm previous reports (Gershoy, 1934; Baker, 1949c and 1957; and Clausen, 1964). Subspecies *praemorsa* is a Pacific coast entity whose range extends inland in the region of the Columbia River and again along the Oregon California boundaries (Figure 20). In both of these inland areas it overlaps the ranges of *V. vallicola* subspecies *vallicola*, *V. bakeri*, subspecies *flavovirens*, and subspecies *linguaefolia*.

Subspecies *flavovirens* represents a morphologically distinct taxon which occurs in isolated pockets in the Rocky Mountains within the range of subspecies *linguaefolia* (Figure 22). Counts of  $2N = 36$  and 48 are the first reported chromosome numbers for this taxon (Table 5).

The previous reports of chromosome counts for *linguaefolia* of  $n = 18$  (Clausen, 1964) and  $n = 24$  (Davidse, 1976) were both confirmed for ten populations in Washington, Oregon, California, Idaho, Montana, and Alberta (Table 5). In all cases pollen viability estimates were high (80 to 95%, Figure 2). Clausen and Baker (Baker, 1949c) first reported a chromosome count of  $2N = 36$  for two populations of *linguaefolia* from Idaho and Utah (Clausen, 1964), and  $2N = 48$  for four populations of *major sensu* Baker. Even though "the morphological characters are not highly distinct," Clausen retained *linguaefolia* at species level, because of his belief of its unique chromosome number. Davidse (1976), finding only counts of  $2N = 48$ , preferred to consider *linguaefolia* in synonymy with *V. praemorsa* subspecies *major sensu* Baker. In nine populations from



Utah, Nevada, and Idaho, all plants had  $n = 24$  with regular meiosis and high pollen fertility. No reliable morphological characteristics or geographic distributions could be correlated with ploidy level (Figure 13, Appendix 4), so it was not considered justifiable to recognize other infraspecific taxa.

Subspecies */inguaefolia* is certainly the most morphologically heterogenous taxon in the species. Considering the areas of sympatry, and chromosome complement similarities, assumptions of genetic isolation between the three *praemorsa* subspecies are not justified. In fact, the possibility of hybridization between these taxa and species of other sections or subsections (Table 2, Table 5) have been reported.

Subspecies */inguaefolia* appears to contain two flavonoid forms (Table 7). The first, and apparently most widespread, resembles the general diverse pattern as defined above. The second is a much reduced profile containing primarily kaempferol (1 and 9) and quercetin (6, 7, 8, and 27) derivatives. The ubiquitous luteolin and apigenin derivatives of *bakeri* and *praemorsa* were completely absent in these specimens, which contained only seven flavonoids including three novel compounds (1, 10 and 27), but did not display any unique morphological features (Figure 13). The possibility exists that these individuals represent ecologically isolated populations which subsequently underwent a reduction of flavonoid profile (Wolf and Denford, 1983). More complete flavonoid surveys may reveal geographic isolation at high elevations or high latitudes at the northeastern edge of the range of *praemorsa*. Certainly it was impossible to establish any correlations based on the meager sampling of only four populations, though these were all collected on or near the continental divide (Table 4).

The diversity of flavonoids present in the leaves of *V. bakeri* and *V. praemorsa*, the extent of morphological variation and the existence of hybrids lead to the hypothesis that these taxa are the most recent in the *V. nuttallii* complex. In only a few scattered populations of */inguaefolia* was it possible to observe flavonoid reduction (Wolf and Denford, 1983) in the elimination of all flavone biosynthesis.

Glaciation events during the Pleistocene likely had an effect on speciation in the Nuttallianae by drastically effecting the climatic conditions on the west coast. At some point in the evolutionary history of these taxa, two lineages arose. *V. vallicola*



and *V. nuttallii*, probably the oldest taxa in the Nuttallianae, differentiated and became established in the more xeric habitats of the valley bottoms in the rainshadows of the Cascade and Rocky Mountains. They can be differentiated from the other Nuttallianae by their minute puberulence, nearly entire leaf margins, seeds with flattened caruncles which cover the funiculus and exceed it in length, and reduced flavonoid profiles consisting of apigenin, kaempferol and quercetin derivatives.

*V. tomentosa*, *V. bakeri*, and *V. praemorsa* became adapted to the more mesic forest habitats of the coastal and montane forests. These taxa, though of diverse morphology, generally possess long pubescence, seeds with globose caruncles which do not cover or exceed the funiculus in length, and diverse flavonoid profiles consisting of apigenin, kaempferol and luteolin compounds. Hybridization between these taxa and taxa of the subsections Chrysanthae and Purpurea indicated that the phylogenetic relationship of the Nuttallianae to other subsections is through these species, as proposed by Clausen (1929). Diploids, tetraploids, hexaploids and octoploids alike have expanded into previously glaciated areas.

Ploidy level in the Nuttallianae has been correlated with morphology, flavonoid chemistry, and phytogeographic data. A classification has been formulated based on these data, which proposes that the *nuttallii* complex be split into eight taxa - five species and three subspecies. This classification, which takes into account the polyploid nature of the complex, attempts to incorporate events of genetic isolation, hybridization, and introgression into the evolutionary history of the group. Correlation of some morphological characters and the presence of different flavonoid profiles support the speculation that two groups of species differentiated from a single ancestral complex. The first group includes *V. vallicola* and *V. nuttallii*, two of the oldest members of the Nuttallianae, which represent homogenous species of wide-ranging distribution. The second group comprises the remaining three species in the Nuttallianae. *V. tomentosa* and *V. bakeri*, also of homogenous morphology, display much more restricted distributions. *V. praemorsa* surely represents the most difficult systematic problem remaining in the complex due to its heterogenous nature and widespread distribution. Hypothetical relationships advanced in this discussion await more in depth analysis into the



morphological, biochemical, and cytological aspects of *V. praemorsa* as well as breeding experiments for confirmation.



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## VI. APPENDIX 1. Data for TAXMAP analyses.

The raw data for all 95 OTUs (Table 4) and 50 attributes (Table 3), is listed as arranged for the TAXMAP data file. The first line represents the FORTRAN format statement.







## VII. APPENDIX 2. TAXMAP Analysis 1.

This analysis was performed using the author's collection of 59 OTUs scored for 50 attributes including flavonoid chemistry, cytological and morphological characteristics. TAXMAP was ran using an equally weighted analysis which does not take into account the relative information content of each attribute.

The TAXMAP print out includes the complete proximity table of the relative distance of each OTU from every other OTU. The nearest neighbour table lists the eight closest neighbours of each OTU in order with their distances. The table summarizing the cluster analysis lists the clusters, cluster members, and single-membered clusters. The Mapping Aids includes the intercluster distances as center-to-center distances and minimum intercluster discontinuities.



PROXIMITIES (AS DISTANCE * 1000), COMPUTED FOR ATTRIBUTES		1	10	50	100	28	11	54	12	30	13	60	14	39	15	81	16	79
1	2	31	3	61	4	47	5	30	6	33	7	43	8	25	9	39	10	28
1	17	217	18	200	19	166	20	132	21	165	22	162	23	140	24	291	25	265
1	32	214	33	283	34	337	35	411	36	279	37	536	38	348	39	314	40	364
1	47	330	48	107	49	69	50	41	51	24	52	24	53	30	54	26	55	23
2	5	27	6	28	7	24	8	27	9	42	10	21	11	51	12	25	13	54
2	20	123	21	138	22	191	23	134	24	287	25	290	26	275	27	266	28	274
2	25	435	36	301	37	562	38	493	39	323	40	405	41	348	42	471	43	400
2	50	41	51	20	52	43	53	36	54	24	55	41	41	42	42	47	431	44
3	9	91	10	90	11	66	12	98	13	93	14	95	15	121	16	106	17	194
3	24	281	25	275	26	308	27	304	28	294	29	311	30	268	31	229	32	258
3	39	374	40	393	41	403	42	519	43	341	44	416	45	417	46	433	47	331
3	54	105	55	96	56	85	57	310	58	338	59	230	55	31	6	36	7	49
4	14	39	15	87	16	110	17	266	18	206	19	178	20	141	21	177	22	210
4	29	243	30	244	31	205	32	185	33	397	34	317	35	415	36	281	37	549
4	44	408	45	361	46	417	47	324	48	112	49	95	50	46	51	39	52	46
4	59	226	6	19	7	30	8	21	9	20	10	19	11	49	12	29	13	41
5	20	119	21	151	22	192	23	131	24	264	25	270	26	255	27	248	28	253
5	35	431	36	295	37	551	38	489	39	321	40	391	41	336	42	468	43	393
5	50	45	51	20	52	37	53	35	54	28	55	29	56	35	57	260	58	271
6	12	35	13	34	14	43	15	67	16	93	17	233	18	201	19	154	20	126
6	27	250	28	254	29	253	30	258	31	252	32	196	33	420	34	350	35	435
6	42	466	43	396	44	421	45	365	46	427	47	345	48	105	49	88	50	41
6	57	267	58	282	59	238	8	35	9	46	10	28	11	49	12	31	13	60
7	20	115	21	148	22	196	23	149	24	284	25	306	26	287	27	275	28	287
7	35	454	36	310	37	566	38	509	39	342	40	420	41	355	42	491	43	419
7	50	45	51	23	52	40	53	30	54	19	55	33	56	43	57	290	58	287
8	14	32	15	77	16	87	17	224	18	212	19	161	20	113	21	148	22	177
8	29	259	30	267	31	229	32	207	33	415	34	344	35	429	36	300	37	551
8	44	425	45	371	46	434	47	338	48	100	49	83	50	44	51	21	52	34
8	59	242	10	31	11	68	12	35	13	35	14	33	15	72	16	97	17	249
9	24	262	25	262	26	245	27	244	28	240	29	240	30	248	31	207	32	188
9	39	315	40	375	41	334	42	463	43	393	44	411	45	359	46	423	47	340
9	54	42	55	38	56	43	57	255	58	263	59	227	61	15	12	31	13	41
10	20	124	21	143	22	184	23	138	24	282	25	285	26	269	27	265	28	263
10	35	438	36	306	37	558	38	496	39	323	40	395	41	343	42	466	43	396
10	50	36	51	21	52	41	53	36	54	36	53	36	54	45	47	48	50	44
11	17	216	18	192	19	197	20	148	21	177	22	158	23	163	24	149	25	177
11	32	246	33	391	34	313	35	409	36	254	37	577	38	469	39	346	40	429
11	47	314	48	123	49	77	50	80	51	46	52	48	53	63	54	64	55	64
12	15	95	16	95	17	245	18	217	19	166	20	119	21	160	22	185	23	134
12	30	270	31	227	32	219	33	409	34	340	35	425	36	297	37	548	38	485
12	45	372	46	420	47	341	48	97	49	91	50	49	51	24	52	26	308	40
13	14	48	15	69	16	100	17	251	18	196	19	173	20	137	21	166	22	186
13	29	251	30	252	31	227	32	204	33	422	34	349	35	434	36	298	37	556
13	44	405	45	372	46	439	47	349	48	177	49	98	50	48	51	44	52	55
13	59	248	15	74	16	93	17	245	18	210	19	167	20	116	21	167	22	198
14	29	246	30	254	31	215	32	206	33	414	34	334	35	424	36	300	37	547
14	44	419	45	368	46	424	47	344	48	103	49	84	50	48	51	27	318	39
14	59	237	16	136	17	221	18	189	19	149	20	107	21	143	22	192	23	123
15	30	248	31	214	32	170	33	385	34	320	35	404	36	254	37	515	38	471
15	45	345	46	412	47	325	48	134	49	120	50	76	51	44	52	55	53	58
16	17	240	18	210	19	231	20	185	21	215	22	203	23	189	24	167	25	198
16	32	275	33	431	34	405	35	440	36	348	37	567	38	447	39	383	40	415
16	47	408	48	151	49	95	50	109	51	92	52	93	53	99	54	116	55	128
17	20	168	21	137	22	76	23	131	24	369	25	331	26	386	27	355	28	372
17	35	351	36	251	37	503	38	457	39	372	40	412	41	387	42	508	43	572
17	50	249	51	241	52	246	53	265	54	242	55	252	56	245	57	383	58	383



18	24	335	25	309	26	373	27	367	28	328	29	355	30	241	31	274	32	288	33	266	34	276	35	309	36	230	37	475	38	422				
18	39	351	40	386	41	389	42	479	43	341	44	393	45	405	46	409	47	296	48	266	49	160	50	220	51	207	52	214	53	249				
18	54	279	55	257	56	208	57	357	58	361	59	259	20	106	21	70	22	111	23	46	24	324	25	295	26	170	27	287	28	281				
18	19	29	281	30	243	31	277	32	217	33	317	34	283	35	374	36	263	37	483	38	475	39	295	40	385	41	315	42	437	43	401			
19	19	44	398	45	354	46	410	47	324	48	226	49	223	50	178	51	169	52	162	53	190	54	180	55	170	56	170	57	290	58	286			
19	19	59	246	21	76	22	126	23	72	24	300	25	305	26	269	27	287	28	281	29	277	30	290	31	250	32	236	33	333	34	270			
20	22	35	379	36	240	37	481	38	470	39	288	40	357	41	306	42	421	43	375	44	384	45	343	46	404	47	293	48	175	49	177			
20	22	50	125	51	111	52	122	53	119	54	126	55	134	56	121	57	293	58	296	59	272	60	260	61	243	62	359	63	337	64	346			
21	21	27	332	28	314	29	337	30	295	31	313	32	267	33	359	34	322	35	404	36	291	37	527	38	514	39	343	40	416	41	361			
21	21	42	469	43	426	44	435	45	393	46	450	47	352	48	208	49	219	50	161	51	149	52	167	53	160	54	170	55	193	56	157			
21	21	57	332	58	324	59	255	23	83	24	349	25	320	26	376	27	380	28	353	29	374	30	281	31	289	32	312	33	270	34	286			
22	22	35	325	36	235	37	490	38	434	39	359	40	384	41	384	42	492	43	342	44	404	45	419	46	423	47	293	48	238	49	155			
22	22	50	201	51	192	52	184	53	216	54	239	55	241	56	201	57	369	58	368	59	260	60	243	61	304	62	308	63	305	64	290			
23	23	29	303	30	260	31	273	32	234	33	320	34	285	35	365	36	268	37	490	38	474	39	308	40	378	41	330	42	445	43	393			
23	23	44	396	45	357	46	421	47	320	48	190	49	190	50	147	51	131	52	134	53	158	54	177	55	171	56	134	57	294	58	296			
23	23	59	243	25	136	26	116	27	101	28	134	29	138	30	206	31	177	32	185	33	217	34	244	35	339	36	216	37	539	38	417			
24	24	39	367	40	416	41	365	42	524	43	396	44	385	45	395	46	491	47	342	48	266	49	282	50	295	51	278	52	277	53	271			
24	24	54	234	55	242	56	272	57	119	58	86	59	195	26	79	27	80	28	72	29	108	30	118	31	104	32	154	33	268	34	258			
25	25	35	293	36	199	37	448	38	426	39	370	40	394	41	393	42	511	43	416	44	422	45	360	46	441	47	394	48	219	49	277			
25	25	50	278	51	288	52	281	53	294	54	260	55	271	56	264	57	97	58	82	59	91	60	91	61	27	63	62	69	63	114	64	192	65	141
26	26	32	161	33	295	34	243	35	309	36	221	37	465	38	435	39	319	40	403	41	360	42	476	43	426	44	317	45	317	46	441			
26	26	47	369	48	222	49	305	50	258	51	271	52	270	53	249	54	215	55	225	56	253	57	83	58	86	59	130	60	62	61	29	90		
27	27	30	176	31	122	32	114	33	329	34	269	35	350	36	234	37	494	38	468	39	360	40	426	41	383	42	504	43	458	44	414			
27	27	45	354	46	493	47	387	48	224	49	310	50	269	51	264	52	272	53	264	54	215	55	226	56	255	57	73	58	86	59	125			
28	28	29	104	30	146	31	142	32	129	33	301	34	244	35	320	36	203	37	479	38	455	39	346	40	432	41	395	42	473	43	434			
28	28	44	393	45	343	46	477	47	358	48	201	49	311	50	247	51	263	52	269	53	265	54	236	55	247	56	95	57	95	58	89			
28	28	59	135	30	148	31	139	32	116	33	318	34	262	35	321	36	223	37	464	38	480	39	367	40	444	41	376	42	509	43	464			
29	29	44	419	45	363	46	493	47	393	48	209	49	308	50	267	51	263	52	251	53	261	54	226	55	235	56	243	57	89	58	107			
29	29	59	146	31	90	32	142	33	286	34	280	35	299	36	219	37	454	38	411	39	357	40	398	41	358	42	492	43	404	44	405			
30	30	45	349	46	423	47	376	48	270	49	263	50	271	51	266	52	259	53	258	54	223	55	239	56	239	57	160	58	137	59	73			
31	31	32	124	33	286	34	260	35	298	36	230	37	413	38	379	39	315	40	344	41	345	42	456	43	358	44	363	45	318	46	399			
31	31	47	337	48	247	49	225	50	229	51	224	52	224	53	234	54	200	55	208	56	233	57	126	58	155	59	112	60	325	61	34			
32	32	35	342	36	211	37	486	38	473	39	362	40	397	41	349	42	469	43	433	44	386	45	326	46	456	47	358	48	205	49	257			
32	32	50	214	51	204	52	218	53	202	54	189	55	205	56	218	57	286	58	328	59	153	60	120	61	349	62	340	63	365	64	309			
33	33	39	330	40	336	41	363	42	426	43	355	44	353	45	315	46	391	47	306	48	367	49	370	50	422	51	407	52	407	53	427			
33	33	54	370	55	383	56	420	57	264	58	303	59	216	60	35	61	379	62	360	63	390	64	321	65	331	66	316	67	48	68	557			
34	34	44	289	45	254	46	395	47	244	48	287	49	349	50	358	51	329	52	327	53	341	54	278	55	299	56	326	57	220	58	240			
34	34	59	219	36	91	37	402	38	317	39	356	40	373	41	389	42	430	43	375	44	374	45	342	46	404	47	332	48	385	49	379			
35	35	50	437	51	425	52	424	53	447	54	381	55	393	56	435	57	286	58	328	59	225	60	345	61	340	62	320	63	355	64	304			
36	36	42	426	43	334	44	334	45	327	46	271	47	408	48	214	49	281	50	276	51	284	52	283	53	280	54	280	55	289	56	293			
36	36	57	193	58	215	59	163	60	305	61	313	62	299	63	41	64	276	65	307	66	324	67	257	68	242	69	316	70	431					
37	37	50	549	51	561	52	542	53	598	54	583	55	568																					



NEAREST NEIGHBOURS (DISTANCE \* 1000)

1	1-572	v-a	53	30
2	2-4-524	v-a	2	2
3	3-9-550	v-a	6	27
4	4-15-567	v-a	8	87
5	5-34-516	v-a	3	39
6	6-35-517	v-a	5	40
7	7-36-519	v-a	11	12
8	8-37-520	v-a	13	34
9	9-38-523	v-a	14	35
10	10-54d-533	v-a	15	35
11	11-56-551	v-a	16	35
12	12-57-579	v-s	17	35
13	13-58-570	v-s	18	35
14	14-60-604	v-s	19	35
15	15-60-556	v-a	20	35
16	16-25-545	v-a	21	35
17	17-22-589	v-bc	22	35
18	18-23-487	v-bc	23	35
19	19-24-488	v-bc	24	35
20	20-50-471	v-o	25	35
21	21-52-486	v-bc	26	35
22	22-53-507	v-bc	27	35
23	23-21-499	v-bc	28	35
24	24-6a-570	n-a	29	35
25	25-8-538	n-a	30	35
26	26-13-571	n-a	31	35
27	27-16a-573	n-a	32	35
28	28-17-576	n-a	33	35
29	29-54a-537	n-a	34	35
30	30-59-596	n-s	35	35
31	31-26-253	n-a	36	35
32	32-27-531	n-a	37	35
33	33-62-622	1-i	38	35
34	34-63-631	1-m	39	35
35	35-64-648	1-a	40	35
36	36-65-654	1-a	41	35
37	37-51-478	m-w	42	37
38	38-40-375	m-w	43	37
39	39-41-394	m-w	44	37
40	40-42-396	b-w	45	37
41	41-43-398	m-w	46	37
42	42-44-414	p-w	47	37
43	43-45-442	m-o	48	37
44	44-46-443	o-o	49	37
45	45-47-458	a-o	50	37
46	46-48-459	m-c	51	37
47	47-49b-463	m-c	52	37
48	48-5b-548	v-a	53	37
49	49-7-568	v-a	54	37
50	50-12-565	v-a	55	37
51	51-14-566	v-a	56	37
52	52-30-547	v-a	57	37
53	53-31-276	v-a	58	37
54	54-29-268	v-a	59	37
55	55-16b-194	v-a	60	37
56	56-28-256	v-a	61	37
57	57-5a-549	n-a	62	37
58	58-55-546	n-a	63	37
59	59-2-112	n-a	64	37



## MAP CLUSTER ANALYSIS: -NUTTALLIANAE, SEPTEMBER, 1983; PLUS HERBARIUM SPECIMENS

(MINIMUM NUCLEUS 0.080, MAXIMUM DROP 0.0439 BOTH ARE 100% OF NORMAL)  
EQUALLY WEIGHTED ATTRIBUTES

CLUS	OTU	OTU DIST	OTU TER.	AVGOF NOS	BEST LINK	NEW LINK	FAR LINK	DROP IN AVG.	FAR OTU	FLAG	NAME OF OTU			
1	53	0.01	54	0.021	0.007	53	0.02	58	0.08	25	0.087-0.000	55-546 n-a		
	8	0.02	54	0.029	0.008	8	0.04	24	0.09	58	0.116-0.029	6a-570 n-a		
	7	0.02	54	0.029	0.008	36-519	v-a	29	0.09	57	0.108-0.008	54a-537 n-a		
	5	0.02	8	0.029	0.000	53	0.04	32	0.09	57	0.138-0.031	27-531 n-a		
	6	0.02	5	0.033	0.004	53	0.04	59	0.09	25	0.129-0.009	2-112 n-a		
	10	0.02	5	0.027	0.006	53	0.04	30	0.07	59	0.150-0.021	59-596 n-s		
	51	0.02	5	0.025	0.002	53	0.03	31	0.09	30	0.131-0.020	24 0.18 26-253 n-a		
	2	0.02	51	0.026	0.002	53	0.04	4-524	v-a	36	0.16	59 0.211 0.080 27 0.23 1204 65-654 1-a		
	9	0.02	5	0.035	0.009	7	0.05	38-523	v-a					
	55	0.02	53	0.034	0.000	10	0.04	16b-194	v-a					
	4	0.02	9	0.043	0.008	53	0.06	15-567	v-a					
	1	0.02	55	0.032	0.011	54	0.05	1-572	v-a					
	12	0.02	51	0.033	0.000	53	0.04	57-579	v-s					
	14	0.02	12	0.034	0.001	6	0.04	60-604	v-s					
	52	0.02	1	0.037	0.003	4	0.05	30-547	v-a					
	50	0.03	51	0.045	0.008	52	0.05	12-565	v-a					
	56	0.03	51	0.045	0.000	53	0.06	28-256	v-a					
	13	0.03	6	0.050	0.006	54	0.07	58-590	v-s					
	11	0.05	51	0.059	0.009	13	0.08	56-551	v-a					
	3	0.06	1	0.089	0.030	54	0.11	9-550	v-a					
	49	0.05	3	0.091	0.002	55	0.12	7-568	v-a					
	15	0.07	6	0.086	0.005	3	0.12	10-556	v-a					
	48	0.08	56	0.112	0.027	49	0.17	5b-548	v-a					
	16	0.08	1	0.103	0.009	48	0.15	25-545	v-a					
	20	0.11	15	0.133	0.030	16	0.19	200	50-471	v-o				
2	21					52-486	v-bc	1	1	11	1	21 31 4 41 2		
	23	0.04				21-499	v-bc	2	1	12	1	22 32 4 42 10		
	19	0.05	23	0.059	0.015	21	0.07	24-488	v-bc	3	1	13	1 33 3 43 11	
	20	0.07	23	0.085	0.026	19	0.11	50-471	v-o	4	1	14	1 24 4 34 3 45 13	
	22	0.08	23	0.105	0.020	20	0.13	53-507	v-bc	5	1	15	1 25 4 35 3 36 3	
	18	0.06	22	0.111	0.006	20	0.15	23-487	v-bc	6	1	16	1 26 4 36 3 46 14	
	17	0.08	22	0.121	0.011	20	0.17	22-489	v-bc	7	1	17	2 27 4 37 5 47 15	
	15	0.11	20	0.161	0.040	17	0.22	204	10-556	v-a	8	1	18	2 28 4 38 6 48 1
3	34					63-631	l-m	9	1	19	2	29 4 39 7 49 1		
	36	0.06				65-654	l-a	10	1	20	2	30 4 40 8 50 1		
	33	0.08	36	0.083	0.023	34	0.09	62-622	l-1					
	35	0.08	33	0.101	0.017	34	0.13	64-648	l-a					
	59	0.16	36	0.206	0.105	35	0.23	1200	2-112	n-a				
4	27					16a-573	n-a							
	28	0.06				17-576	n-a							
	26	0.06	27	0.067	0.004	28	0.07	13-571	n-a					
	25	0.07	28	0.078	0.011	27	0.08	8-538	n-a					
	57	0.07	27	0.088	0.010	25	0.10	5a-549	n-a					







### VIII. APPENDIX 3. TAXMAP Analysis 2.

TAXMAP analysis 2 contained 72 OTUs including 13 OTUs representing typespecimens (holotypes, isotypes, and paratypes) along with the 59 OTUs used for TAXMAP analysis 1 (Appendix 2). A weighted analysis employed the relative information content of the attributes to calculate the relative inter-OTU distances.

Only the TAXMAP cluster analysis is listed here.



MAP CLUSTER ANALYSIS:-NUTTALLIANAE, 1983: 72 OTUS, ATT 1-50, weighted

(MINIMUM NUCLEUS 0.102, MAXIMUM DROP 0.0400 BOTH ARE 100% OF NORMAL)

CLUS	OTU	DIST	OTU	AVGOF	DROP	FAR	DIST	FLAG	NAME OF OTU
TER	NOS	BEST	BEST	NEW	IN	OTU	FAR		
NO.		LINK	LINK	LINKS	Avg.		OTU		

1	53						31-276	v-a	
	54	0.03					29-268	v-a	
	7	0.03	54	0.042	0.017	53	0.05	36-519	v-a
	2	0.03	7	0.045	0.003	53	0.06	4-524	v-a
	51	0.03	2	0.042	-0.004	53	0.05	14-566	v-a
	10	0.03	2	0.044	0.002	53	0.06	54d-533	v-a
	5	0.03	10	0.043	-0.000	53	0.06	34-516	v-a
	6	0.03	5	0.048	0.004	53	0.07	35-517	v-a
	9	0.03	5	0.055	0.007	54	0.08	38-523	v-a
	8	0.03	5	0.039	-0.016	7	0.05	37-520	v-a
	1	0.03	51	0.046	0.007	7	0.06	1-572	v-a
	52	0.03	1	0.057	0.011	6	0.07	30-547	v-a
	4	0.03	9	0.067	0.011	53	0.10	15-567	v-a
	12	0.03	8	0.047	-0.021	53	0.07	57-579	v-s
	14	0.03	12	0.052	0.005	4	0.07	60-604	v-s
	55	0.04	53	0.064	0.012	4	0.09	16b-194	v-a
	64	0.04	54	0.115	0.051	4	0.21	1000	S8530 type-s
2	13						58-590	v-s	
	56	0.05					28-256	v-a	
	52	0.05	56	0.064	0.012	13	0.07	4	30-547 v-a
		LINK TO CLUSTER	-1						
3	34						63-631	1-m	
	36	0.06					65-654	1-a	
	33	0.07	34	0.071	0.010	36	0.07	62-622	1-i
	70	0.08	33	0.107	0.036	34	0.14	CFB225	type-g
	69	0.08	70	0.146	0.039	36	0.19	AN5481	type-e
	35	0.09	33	0.108	-0.038	69	0.12	64-648	1-a
	47	0.10	70	0.180	0.071	35	0.25	1000	49b-463 m-c
4	3						9-550	v-a	
	50	0.06					12-565	v-a	
	7	0.06	50	0.077	0.014	3	0.09	4	36-519 v-a
		LINK TO CLUSTER	-1						
5	21						52-486	v-bc	
	23	0.07					21-499	v-bc	
	19	0.07	23	0.087	0.021	21	0.11	24-488	v-bc
	62	0.07	23	0.082	-0.006	21	0.09	AN4340	type-v
	10	0.07	62	0.113	0.031	19	0.15	4	54d-533 v-a
		LINK TO CLUSTER	-1						
6	24						6a-570	n-a	
	58	0.07					55-546	n-a	
	25	0.08	58	0.100	0.031	24	0.12	8-538	n-a
	28	0.08	25	0.111	0.010	24	0.14	17-576	n-a
	27	0.08	28	0.092	-0.018	58	0.10	16a-573	n-a
	57	0.08	27	0.098	0.006	28	0.12	5a-549	n-a
	32	0.08	57	0.118	0.020	24	0.14	27-531	n-a
	26	0.09	25	0.111	-0.007	32	0.16	13-571	n-a
	59	0.09	57	0.111	-0.000	24	0.15	2-112	n-a
	29	0.10	25	0.120	0.009	59	0.15	54a-537	n-a
	30	0.10	59	0.136	0.016	26	0.19	59-596	n-s
	31	0.10	25	0.120	-0.016	24	0.14	26-253	n-a
	67	0.11	59	0.167	0.046	28	0.21	1000	ANSPH type-n



7	16								25-545	v-a
	49	0.08							7-568	v-a

\*\* NEEDED .GT. FOUND FOR NEXT OTU \*\*

	12	0.08	49	0.355	0.273	72	0.63	1004	57-579	v-s
-----										
8	64								S8530	type-s
	66	0.09							RS-BB	type-r

\*\* NEEDED .GT. FOUND FOR NEXT OTU \*\*

	54	0.09	66	0.359	0.273	72	0.63	1004	29-268	v-a
-----										
9	17								22-489	v-bc
	22	0.09							53-507	v-bc
	18	0.10	17	0.099	0.008	22	0.10		23-487	v-bc
	65	0.10	17	0.152	0.053	18	0.23	1000	CFB67	type-ph
-----										
10	11								56-551	v-a
	48	0.10							5b-548	v-a

\*\* NEEDED .GT. FOUND FOR NEXT OTU \*\*

	9	0.10	48	0.364	0.268	72	0.63	1004	38-523	v-a
-----										
11	15								10-556	v-a
	20	0.10							50-471	v-o
	64	0.10	20	0.135	0.033	15	0.17	4	S8530	type-s
	LINK TO CLUSTER -8									
-----										

#### ISOLATED OTU'S (SINGLE MEMBER CLUSTERS)

##### CLUSTER OTU LABEL

12	37	51-478	f-w
13	38	40-375	m-w
14	39	41-394	m-w
15	40	42-396	b-w
16	41	43-398	m-w
17	42	44-414	p-w
18	43	45-442	m-o
19	44	46-443	o-o
20	45	47-458	a-o
21	46	48-459	m-c
22	47	49b-463	m-c
23	60	B9634	1type-p
24	61	H00K/k	type-m
25	63	S10200	type-x
26	65	CFB67	type-ph
27	67	ANSPH	type-n
28	68	B8662	type-o
29	71	H3156	type-f
30	72	SMH222	ctype-f

##### CLUSTER MEMBERSHIP INDEX

1	1	11	10	21	5	31	6	41	16	51	1	61	24	71	29
2	1	12	1	22	9	32	6	42	17	52	1	62	5	72	30
3	4	13	2	23	5	33	3	43	18	53	1	63	25		
4	1	14	1	24	6	34	3	44	19	54	1	64	8		
5	1	15	11	25	6	35	3	45	20	55	1	65	26		
6	1	16	7	26	6	36	3	46	21	56	2	66	8		
7	1	17	9	27	6	37	12	47	22	57	6	67	27		
8	1	18	9	28	6	38	13	48	10	58	6	68	28		
9	1	19	5	29	6	39	14	49	7	59	6	69	3		
10	1	20	11	30	6	40	15	50	4	60	23	70	3		



#### IX. APPENDIX 4. TAXMAP Analysis 3.

TAXMAP analysis 3 contained 39 OTUs 17 OTUs from the author's collection, 8 OTUs from cytologically known herbarium specimens which were extracted for flavonoid profile analysis, and 8 type specimens. In an attempt to clarify the relationships between *V. praemorsa*, *bakeri* and *linguaefolia*, all specimens of *V. vallicola* and *V. nuttallii* were removed from this analysis.

A weighted analysis was performed using attributes 1 to 50. The resulting output is given in the form of a cluster analysis table. A second TAXMAP analysis was performed using only the morphological attributes, 30 to 50. The resulting output from the morphological analysis is also given as a cluster analysis table.



MAP CLUSTER ANALYSIS:-NUTTALLIANAE, PRAEMORSA: 39 OTUS, ATT 1-50, WEIGHTED  
(MINIMUM NUCLEUS 0.180, MAXIMUM DRDP 0.0318 BOTH ARE 100% OF NDRML)

CLUS	OTU	DIST	OTU	AVGDF	DRDP	FAR	DIST	FLAG	NAME	OF	DTU
TER	NOS	BEST	BEST	NEW	IN	DTU	FAR				
NO.	LINK	LINK	LINK	LINK	LINK	LINK	LINK				
<hr/>											
1	2				63-631	1-m					
4	0.07				65-654	1-a					
	1	0.08	4	0.084	0.012	2	0.09				
37	0.09	1	0.115	0.031	2	0.15					
36	0.09	37	0.162	0.047	4	0.20					
	3	0.10	4	0.124-0.038	2	0.15					
15	0.10	37	0.212	0.088	3	0.32	1000	49b-463	m-c		
<hr/>											
2	22				B8388	b-c					
28	0.09				B8052	b-c					
25	0.10	28	0.120	0.031	22	0.14					
23	0.12	28	0.150	0.030	22	0.18					
					B8377	b-c					
					B5215	bg-c					
37	0.13	23	0.206	0.056	22	0.26	1004	CFB225	type-g		
<hr/>											
3	19				B8385	a-c					
29	0.10				B11462	1type-a					
30	0.10	29	0.126	0.031	19	0.15					
					B12086	1type-a					
15	0.13	29	0.185	0.058	30	0.22	1000	49b-463	m-c		
<hr/>											
4	31				B7408	m-1					
35	0.10				B8662	type-o					
32	0.11	31	0.150	0.053	35	0.19					
27	0.11	32	0.141-0.008	31	0.16						
					GD1640	m-n					
37	0.11	32	0.175	0.033	35	0.22	4	CFB225	type-g		
5	11				45-442	m-o					
	12	0.14			46-443	o-o	typesite				
37	0.16	11	0.159	0.022	12	0.16	4	CFB225	type-g		
6	20				B8403	b-c					
24	0.16				B8406	b-c					
13	0.16	20	0.199	0.042	24	0.24	1000	47-458	a-o	typesite	
7	6				40-375	m-w					
26	0.16				D1756	m-1					
5	0.17	26	0.218	0.055	6	0.26	1000	51-478	m-w		
<hr/>											
8	16				B9634	1type-p					
17	0.17				B8059	p-c					
31	0.19	16	0.203	0.036	17	0.22	1004	B7408	m-1		
9	8				42-396	b-w					

## ISOLATED DTU'S (SINGLE MEMBER CLUSTERS)

## CLUSTER DTU LABEL

CLUSTER	DTU	LABEL
11	7	41-394
12	9	43-398
13	10	44-414
14	13	47-458
15	14	48-459
16	15	49b-463
17	18	0.1738
18	33	88699
19	34	S10200
		type-x

## CLUSTER MEMBERSHIP INDEX

1	1	11	5	21	9	31	4
2	1	12	5	22	2	32	4
3	1	13	14	23	2	33	18
4	1	14	15	24	6	34	19
5	10	15	16	25	2	35	4
6	6	7	16	8	26	7	36
7	7	11	17	8	27	4	37
8	8	18	17	28	2	38	10
9	9	12	19	3	29	3	39
10	13	20	6	30	3		



MAP CLUSTER ANALYSIS:- NUTTALLIANAE. PRAEMORSA: 39 OTUS, ATT 30-50, WEIGHTED



## X. APPENDIX 5. Representative Specimens

*Viola nuttallii*

**CANADA, Alberta:** *D Fabijan* 570 (ALTA), N bank of Bow River, Scandia, 2400'; 538 (ALTA), Cochrane Hill, Cochrane, 4000'; 571 (ALTA), N bank Old Man River, Taber, 2500'; 573 (ALTA), Etzikom Coulee 38.6 km S of Taber on hwy 36, 3100'; 576 (ALTA), Writting-On-Stone, 3000'; 537 (ALTA), Nose Hill, Calgary, 3700'; 253 (ALTA), Fabyan, 2100'; 531 (ALTA), 18 km N of Wainwright on hwy 41, 2000'; 549 (ALTA), 5.7 km E Cluny on hwy 1, 2800'; 546 (ALTA), Turner Valley, 4100'; *C Strand* 18 (ALTA, DAO), 2 mi S of Pearce, Old Man River Valley; *EH Moss* 1107 (ALTA, DAO, G), S of Lethbridge; *MG Dumais* 6054 (ALTA), W of Wainwright at Battle River Campsite, hwy 14; 6514 (ALTA), Drumheller, W side of River; *WC McCalla* 11071 (ALTA), W of MacLeod; 7355 (ALTA), Calgary; 4594 (ALTA, UBC), North Hill, Calgary; 8554 (ALTA, UBC), Riley Park, Calgary; 8557 (G, UBC), N of Riley Park, Calgary; 8576 (G), N of Riley Park, Calgary; 8601 (ALTA, UBC), N of Riley Park, Calgary; 9382 (ALTA, DAO, G, UBC), N of Riley Park, Calgary; 10313 (ALTA, UBC), N of Riley Park, Calgary; 12850 (ALTA, UBC), N of Riley Park, Calgary; *HW Pine* and *C.A. Wallis* V-77 (CAN), 4 mi ENE of Rosebud, 2600'; V-12 (DAO), 1 / 4 mi W of Gatine, 2350'; *HJ Scoggan* 16607 (CAN), Pincher Creek; *ME Moodie* 821 (G, MO, US), Black Birch Coulee, Rosedale, 2200-2500'.

**Saskatchewan:** *D Fabijan* 596 (ALTA), 3.5 km S and 2.3 km W of Wood Mountain Park; *B deVries* 468 (DAO), E of Lebret, Qu'Appelle Valley; *B Boivin* 13450 (DAO), R. Tete-a-la-Biche, 3 mi S of Carievale; *RJ Ledingham* and *R.C. Russell* S4910 (DAO), Moose Jaw; S4916 (DAO), Elbow, Sask. R. Valley; S5036 (DAO), Outlook, towards river; *B.J. Sallans* and *R.C. Russell* (DAO), Fort Qu'Appelle; *L.T. Carmichael* R-14 (DAO), Qu'Appelle Valley; *R.C. Russell* and *W.P. Fraser* (DAO), Sutherland; *B.J. Sallans* and *R.C. Russell* (DAO), Buffalo Pound Lk., Chamberlain; *N.A. Skoglund* 26 (DAO, CAN, G), 35 km SE of Kyle; *R.C. Russell* S4736 (DAO), Katepwe, Qu'Appelle Valley; 2828 (DAO), Katepwa; *G.F. Ledingham* 49-13 (DAO), N of Wascana Pond, Regina; *J.H. Hudson* 1937 (DAO), Mortlach, Moose Jaw district; *G.J. Jones* 634 (DAO), Lake Marguerite, 12 mi S of



Indian Head; *W.H. Cameron* (DAO), Sutherland; *W.P. Fraser* (DAO), Saskatoon.

**Manitoba:** *B. Boivin* 13427 (DAO), 1 mi SE of Goodlands; *G. Stevenson* 431 (DAO), Brandon; *H.H. Marshall* 34 (DAO), Brandon; *T.J.W. Burgess* (DAO), first crossing, Souris River; *G.M. Dawson* 198 (DAO), E crossing, Souris River; *H.J. Scoggan* 7183 (CAN), Souris River, 8 mi N of Minto; *G.A. Stevenson* 766 (CAN), Brandon; *J. Macoun* 12446 (CAN), entrance to Exp. Farm, Brandon; *R.D. Bird* (ALTA), Little Saskatchewan River, Brandon; *B. Boivin* 13429 (DAO,G), W of Dalny, Souris River.

**UNITED STATES: Montana:** *WW Jones* (RM,DS,G,UC,US), Gallatin Co., Bozeman; *E.J. Moore* (RM,DS,US,G), Gallatin Co., Bozeman; *JW Blankinship* 552 (RM,PH,DS,US,UC,MO), Bozeman; (UC,G), Broadwater Co.,Lombard; *Hoag and Mayer* 1 (RM), Decker Quadrangle, Big Horn Co., 3430-3800'; *WE Booth* (RM), 2 mi N of Ryegate, Golden Valley Co.; *LC Ellison* 3301 (RM), Custer Co., 2800'; *AW Collotzi and G Davidse* 647 (CAS,DS,PH,G,US,WS), S of Billings airport, Yellowstone Co.; *HR Flint* 46 (RM), Long Pines Div., Custer Forest, 3800'; *TN Traphagen* (PH), Deer Lodge; *HT and JM Rogers* 1334 (WS), Lambert, Richland Co.; *PH Hawkins* (UC), Absarokee; *ED Wooton* (US), Vicinity of Helena, Clark Co; *J Roseneau* (DAO), coulee near Giant Sprints Rocky area, Great Falls, Cascade Co; *E. Starz* (MO), Helena.

**North Dakota:** *J. Lunne* (DS,RM,PH,MO,US), Leeds, Benson Co; (G), Butte, Benson Co; *W.F. Bergman* 1370 (DS), Enderlin; *E.T. Wherry* (PH), Stark Co., 3.5 mi W Dickinson; *C.L. and M.W. Porter* 10,368a (RM), Billings Co., Medora and T. Roosevelt Nat. Mem. Park, 2500'; *W.F. Bergman* 1434 (PH), Kathyrn; *L.F. Lautenschlager* 206 (CAN), Ward Co.; *J.T. Sarvis* 39 (US), Mandan, Morton Co.; *O.A. Stevens* 2587 (CAN, UC, US), Morton Co., Glen Ullin; (US), Steele; *W. Harken* (US), prairies; *W.N. Suksdorf* (WS), Sedalia; *J.F. Brenkle* (MO), Kulm; *J.F. Bergman* 1370 (MO), Enderlin; *R.P. Williams* 880 (MO), Shell Butte, 13.5 mi S, 3 mi W of Napolean.

**South Dakota:** *W.F. Forwood* 38 (US,CAN), Meade Co., Black Hills near Ft. Meade; *F.L. Bennett* 72 (MO), N Black Hills, N Spearfish; *D. Griffiths* (GH, US), Brown Co., Aberdeen; *J.J. Thornber* (GH, UC, MO), Iroquis; *J.F. Brenkle* 41-06 (GH, MO), Northville; *L.A. Hanna* 141 (MO), Quashnick farm, Artas; *W. Larwence* (WS), Lake Kampesky; *W.H. Over* 2097 (US), Bear Creek, Washabaugh Co.; *P.A. Rydberg* 550 (GH), Hot Springs, 3500'; *J. Murdock* 4060 (GH), Rocky Ridge, Bald Hills, 5200'; *E.T. Wherry* (PH), Fall



R. Co., 8 mi E Smithwick, C.A. Barr 1059 (PH), 8 mi SE Smithwick; R.R. Krebs 62 (RM), Jackson Co., Buffalo Gap Nat. Grassland, 2500'; H.E. Hayward 655 (RM), Badlands Cedar Canyon; 985 (RM), Boulder Cr. Park, 6 mi E Deadwood; 938 (RM), Kermosa; H. Lee 532 (RM), Rapid City; G.M. Dodge 21 (RM), Custer Forest, Eagle's Nest Ranger Station; L.W. Sheving 34.5 (RM), Custer Forest.

**Nebraska:** E.S. Nixon 4 (RM), Sheridan Co., 13 mi N Hay Springs; J.M. Bates (RM), Valentine; R.L. McGregor 19869 (DS), Garden Co., 4 mi S Lewellen; F.V. Hagen (MO), Willow Creek; W.L. Todstead 12 (GH), Holt Co., Niobrara R. valley N of O'Neill; 41 (GH), a mi W of Ohadron; S. Stephens 38080 (GH), Deuel Co., 2 mi N, .5 W Big Springs; S. Stephens and R. Brooks 11587 (GH), Scotts Bluff Co., 2 mi W of Gering; S. Stephens 38062 (GH), Keith Co., 13 mi W Lamoyne.

**Kansas:** L. Watson (GH), Ellis.

**Wyoming:** Hayden (GH), Cheyenne, Wyoming Terr.; M. Cary 315 (US), W of Isley; L. Williams 2185 (UC, US, WS), 1 mi E of Laramie, Albany Co.; C.L. and M.W. Porter 7747 (DAO, UC, CAS), near Rock River, Albany Co.; 7734 (RM), Sybille Cr., Platte Co.; H. Symons 51 (CAN), near Laramie; A. Brebner 5 (CAN), N of Laramie; K. Roach (CAN), Laramie plains; A. Nelson 6826 (CAN, PH, DS, CAS, UC, MO, RM, US, WS, GH), Laramie hills, Albany Co.; 226 (GH, RM), Laramie hills; 6956 (GH, MO, US, RM), Sand Cr., Albany Co.; 209 (UC), Laramie; 37 (GH, US, PH, RM), Laramie hills; 1229 (RM), Laramie; M. Ownbey 544a (UC, WS, RM, DS), 5 mi NW Hulett, Crook Co., 4300'; 544b (UC, RM, DS), 7 mi NW Hulett, Crook Co., 4500'; 583 (MO, UC, WS, DS), 8 mi NW Hulett, Crook Co., 4500'; F. Tweedy 4627 (US), Fort Steele, Carbon Co.; A.A. Beetle 10359 (UC), Boulton Creek Expt. Area, Natrona Co.; J.F. Macbride 2271 (RM), Albany Co., Centennial; B.C. Buffum (RM), Laramie hills; W.G. Solhiem 418 (RM), Laramie hills; B.E. and L. Barsum 1206 (RM), Albany Co., Sheep Mtn, #11 1.9 mi SW of #130, 7800'; J. Finzell 171 (RM), Laramie Co., 7 mi W Cheyenne, 6000'; F.B. Current 242 (RM), Laramie Co., 5 mi NW Cheyenne, 6100'; A. Nelson (RM), Moorcroft; 9614 (RM), Bird's Eye; B. Dickson 273 (RM), Sheridan Co., Big Horn For., 4500'; D.G. Reardon and L.M. Mayor 136 (RM), Sheridan Co., Big Horn coal site; B.E. Nelson 1586 (RM), Niobrara Co., 22.5 mi NW Lusk, 5200'; 1535 (RM), Platte Co., 21 mi N Guernsey, 5350'; C.L. Turner 63 (RM), Converse Co., W of Douglas; E.B. Snel 1 (DS), Buffalo; B. Luce 2 (RM), Goshen Co., Table Mtn Wildlife Unit; D.L. Martin



1520 (RM), Hot Springs Co., Eagles Nest Ranch, 6770'; 1145 (RM), Kirby, Little Sand Draw oilfield; *G.R. Kleeberger* (CAS), Wyo.; *J.F. Macbride* 2720 (RM), Rock River, 7000'; *Cox, Dunn, F. Leak* 1764 (RM), Goshen Co., 1.5 mi N Lingle.

**Arizona:** *L.M. Newlon* 783 (JEPS), Yarapai Co., Ash Fork, 20 mi SW.; *H.H. Rusby* (US), Ash Fork.

**Colorado:** *E.L. Johnston* 8746 (US), Lyons, Boulder Co.; 837 (GH), Trinidad; 886 (GH), Morley; *R.F. Daubenmire* (WS), Boulder Co., Redrocks, Boulder; *Ramely and Richards* 15950 (UC, WS, DS, PH, CAS, RM), Black shales N of Boulder; *C.T. Robbins* 355 (UC), Flagstaff Mtn, ca .5 mi W of Boulder; 514 (UC), Las Aminas Co., E of Delagua; *K.M. and M.C. Wiegand* 1485 (GH), Crouse, Larimer Co.; *C.S. Crandell* 469 (US), Howes Gulch; (US), Dixon Canon; (US, CAN, UC, GH, RM, DS), Horsetooth Gulch; (GH, CAN, US, RM), Bluffs N of La Porte; *J.H. Cowan* (US, RM), Horsetooth Gulch; 59, (MO), Fort Collins; *J.H. Ehlers* 6914 (GH), Lookout Mtn, Golden, Jefferson Co., 5600'; 7961 (GH), Golden; 6788 (GH), Walsenberg, Huerfana Co.; 6774 (GH), Morley, Las Animas Co.; *L.J. Dorr* 209 (MO), Golden, Jefferson Co.; *I.W. Clokey* 4209 (WS, US, PH, CAS, RM), Clear Creek, Jefferson Co.; 2676 (CAN, UC), Prairie, Denver; 2767 (PH, RM), Denver; *R. Duthie and I.W. Clokey* 3819 (CAS, DS, CAN, DAO, GH, PH, MO, US, UC, WS, RM), Wolhurst, Douglas Co.; *J. Wolf* 76(56) (US), Denver; *M. Lamm* 19393 (CAN, RM, PH), Ramah, El Paso Co.; *W.W. Eggleson* 20171 (GH, RM), Branson, Las Animas Co.; *C.M. Rogers* 5656 (US), W end Mesa de Maya; *R.C. Rollins* 2061 (GH, UC, DS), Pueblo Co., 11 mi S of Pueblo, 4700'; *A. Nelson* 11526a (UC, RM), Walsenburg-Pueblo; *R.A. Rydberg and F.K. Vreeland* 5883 (CAN, WS), Cuchara valley, near La Veta, 2100m; 5884 (RM), near Denver; *T.J. Bandergee* (GH), Canon City, 5400'; 42 (MO, UC, PH), Canon City, Colorado Terr.; *H.L. Zobel and C.W. Penland* 1205 (PH, CAS), Deer Creek Canyon, Canyon City Road, 5800'; *J.A. Ewan* 12985 (CAS), Las Animas Co., Berwind Canyon; 11165 (CAS), Boulder Co., Gregory Creek above Boulder; *R.K. Gierisch* 1351 (RM), Clear Creek Co., Rio Grande Forest; *B.H. Smith* 7 (PH), Pagosa Springs; *K.E. Phelps* (CAS), Broomfield; *R.K. Gierisch* 869 (RM), 9 mi N Denver; *C.S. Williamson* (PH), Denver Co.; *J.M. Coulter* (PH), Denver; *E.L. Berthaud* (RM), Golden, Jefferson Co.; *A. Eastwood* 5392 (CAS), Clear Creek Valley, 1587m; *G.E. Osterhaut* 680 (RM), 2-3 mi E Spring Canon; 5238 (RM), Gulch E of Horsetooth Mtn; (RM), Weld Co., New Windsor; (RM), Dale Creek; *B. Hammel* 208 (RM), 3 mi N Livermore



hwy 287, 5600'; *G.S. Dodds* 1979 (RM), Valley-Fossil Creek; *C.F. Baker* 801 (RM, US, MO), Larimer Co.; *W.S. Cooper* 74 (RM), Estes Park, Forks of Big Thompson, 6000'; *F. Tweedy* 5565 (RM), near Boulder; *F. Rama/ey* 1530 (RM), near Boulder; 40 (RM), near Boulder; *A.G. Vesta/* 343 (DS), Boulder; *A.L. Bacon* (PH), Greeley; *M.E. Newlin* (PH), Colorado; *W.A. Henry* 165 (PH, MO), Boulder Canon; *E. Hall and J.P. Harbour* 55 (US, GH), Lat 41°; *H.L. Shantz* 1359 (US), Akron; *E. Palmer* (US), Colo. Terr.; *H.C. Hanson* c482 (MO), Boulder; *F. Nis/zenus* 1169 (MO), Denver.

*Viola vallicola* A. Nels.

**CANADA, British Columbia:** *H. Groh* (DAO), Bridesville; (DAO), Summerland; *J. Bostock* (DAO), Keremeos; (DAO), Summerland; *Hitchins* 1-003 (UBC), Hat Creek, 3300'; *Beamish, Luetiens, Krause* 680015 (CAN), Old Cawston Road, Oliver, 2800'; *Beamish and Vrugthon* 60298 (UBC), Oliver; *W.B. Johnston* (UBC), Newgate; *E. Jacobs* 56 (UBC), Midway; *Spreadborough* 70,877 (CAN), Midway; *D. Morrison* (UBC), Flatiron Mtn; *D.L. Krause* (UBC), 10 mi SW Princeton; *J. Grant* (UBC), Vernon, 1200'; *E.W. Warren* (UBC), Vernon; *E. Wilson* 5274 (UBC), Armstrong, Okanagan; *J.W. Eastman* (UBC), Penticton; *J.A. Calder and D.B.O. Saville* 8125 (DAO), 8 mi SW Okanagan Falls, 3800'; *Calder, J.A. Parmelee and R.L. Taylor* 16793 (DAO), 14 mi N Merritt; 167474 (DAO, UC, US), Stump Lake 25 mi NE Merritt; *W.C. McCalla* 6874 (UBC, ALTA), Cranbrook; *E. Wilson* 15 (WS), Armstrong; *J.R. Anderson* 69 (WS), Grand Forks; *T.T. McCabe* 1975 (UC), 20.5 mi E Kamloops; 1978 (UC), 10.2 mi from Pritchard to Princeton; 5955 (UC), Anarchist Mtn, Osoyoos.

**Alberta:** *W.C. McCalla* 8667 (ALTA), N of Calgary; 11353 (ALTA), Nose Hill, Calgary; 11382 (ALTA), Nose Hill, Calgary; 10383 (ALTA, GH), Nose Hille, Calgary; 8600 (ALTA, CAN), Normal School, Calgary; 3642 (ALTA), Normal School, Calgary; 8573 (ALTA, CAN, DAO), NW of Calgary; 8587 (ALTA, DAO), NW of Calgary; 8587 (ALTA, GH), NW of Calgary; 8567 (ALTA, DAO), King George School, Calgary; 8572 (ALTA), King George School, Calgary; 12210 (ALTA, GH), Highwood River, 6 mi W Longview; 6817 (ALTA, DAO), between Pincher Creek and Cowley; 9386 (ALTA, GH), east of Cowley; 9617 (GH), Nose Hill, Calgary; *J. Macoun* 18201 (CAN), Calgary; *V.C. Brink* (UBC), Scotfield, SE of Hanna;



*D. Hancock* 33 (ALTA), 3 mi S of Aden; *E.H. Moss* 5075 (ALTA), near DeWinton, S of Okotoks; 9777 (ALTA, CAN, DAO), near Elkwater, Cypress Hills; *McCormack* (ALTA), Cypress Hills; *Lewis and Kulyk* 77-5 (CAN), Cypress Hills Prov. Park; *Spreadborough* 3095 (CAN), Medicine Hat; *H.E. Groh* 64 (ALTA), E of Lethbridge; *Dawson* 246 (CAN), N of Milk River; *J.J. Sexsmith* 513 (DAO), Brocket; *F.J. Jenkins* 102703 (CAN), Porcupine Hills; *J.G. Packer* 5064 (ALTA), Maskinonge Lake, Waterton; *R.C. Russell* S5190 (DAO), Elkwater; *R. Kuchar* 2366 (ALTA), Waterton, Blakiston Cr., 4300'; *J. Kuijt* 9 (CAN), Waterton, Bellevue Ridge.

**Saskatchewan:** *R.J. Ledingham* and *R.C. Russell* 4934 (DAO), Cypress Hills, 20 mi S of Maple Creek; S4920 (DAO), Keeler; *R.C. Russell* and *B.J. Sallons* (DAO), Duval, Last Mountain; *B. Rawlinson* and *G. Ledingham* 49-162 (DAO), Pilot Butte, 10 mi E of Regina; *B. Boivin* 13466 (DAO), R. Plumee, 7 mi S of Carnduff; *L. Jenkins* (DAO), Hoosier; *J.H. Hudson* 1933 (DAO), Trewdale; *C. Frankton* 1131 (DAO), 8 mi S, 1 mi W of Saskatoon near Beaver Creek; *R.C. Russell* S4129 (DAO), Aylesbury, valley of Arm River; *D.S.E. Clark* (DAO), Cypress Hills; *R.C. Russell* (DAO), Saskatoon; *W.P. Fraser* (ALTA, DAO), Saskatoon; *J. Looman* (DAO), Mortlach; *G.H. Argus* 3811 (DAO, GH), nwy 5 E Borden on N Saskatchewan R.; *A.W. Anderson* (CAN), Pike Lake; *G.H. Turner* 5597 (ALTA), S of Karen; *J.M. Macoun* 3095 (US), Cypress Hills, NWT; (US, GH), Crane Lake, Assa.; *I.W. Clokey* (UC), Sefton.

**Manitoba:** *B. Boivin* 13429 (ALTA), l'ouest de Dalney, Riviere Souris.

**UNITED STATES, Montana:** *J. Grove* (DAO), 8 mi N of Kalispel; *J.W. Blankinship* 8 (US); (UC, RM), Bozeman; 77 (CAN, DS, PH, MO), Bozeman; 78 (CAN, PH, US), Bozeman; (GH), Custer; *E.O. Wooton* (US), vicinity of Helena; *F.H. Rose* 27 (WS), between Drummond and Garrison; 40 (GH, MO, PH, DS, CAS, UC, WS), Miller Creel valley near Missoula; 55 (DAO, WS), Missoula Co, Send Higgins ave Paddy Cr, Missoula; *C.H. Moore* (MO), near Butte, 5500-6000'; *R.A. Coster* 2 (RM), Beartooth Forest, 6000'; *C.H. Hurst* 285 (RM), Absaroka Forest, 6300'; *P.F. Stickney* 426 (RM), Missoula Co, Mt Jumbo, Lolo Forest, .25 mi E Missoula, 3500'; *J.E. Kirkwood* 1363 (GH, PH, UC), Sentinel MT, S of U, Missoula, 3300'; *P. Cantou* (CAS), Rattlesnake Cr, Bonner; *Dietter and Barkley* 2347 (RM, UC, MO), N slope Mt, Sentinel, 3300'; *B.B. Irwin* 99 (RM), plains about Missoula; *G.E. Osterhaut* 3233a (RM),



Grand Co, Sulphur springs; *H.N. Wheeler* 595 (RM), Sapirnerd; *A. Nelson* 5438 (GH, UC, WS, MO, DS, RM), Madison Co, 10 mi E of Monida; (RM), La Plata Mtns; *C.W. Griffins* (RM), Hodge, Wilbur Co; *W.W. Eggleson* (RM), Grey cliff, Sweet Grass Co, 1190m; *W.E. Booth* 8551 (RM), Big Timber, Sweet Grass Co; 56202 (DAO), Conrad, Pondera Co, 15 mi S; *A. Roemer* 718 (RM), Park Co, Absaroka Forest, Eagle Creek, 5000'; *W.W. Jones* (CAS, US, WS), Bozeman; *J. Williams* 3 (DS), Bozeman, near flour mill; *E.J. Moore* (DS, GH, US), Bozeman; *B.J. Jones* (DS, MO, US), Bozeman; (GH, US), Sedan; *E.P. White* 581 (RM), Gallatin Forest, 6500'; *R.L. Grabhoffer* 7 (RM), Jefferson Forest, Carrless Creek, 6500'; *E.J. Woolfolk* W-12 (RM), Custer Co, US range livestock expt stat, 2400'; *W.M. Laybourn* D8-6 (RM), Jefferson Forest; *E.W. Hartwell* 324 (RM), Custer Forest, La Porte School sect.; 529 (RM), Custer Forest, White tail R. St. Powder R. Co, 3600'; 323 (RM), White tail Ranger Station; *A.W. Vogelsang* D2-16 (RM), Beaverhead Forest; *H.H. Hendron* D4-85 (RM), Lewis and Clark Forest, 4500'; *J.E. Schmartz* JES-311 (RM), Powell Co, Deerlodge, 6450'; *J.H. Thomas* 2623 (RM), Mt Helen, Helena; *H.D. Cook* (GH), Bozeman; *J. Kinney* (US), Bozeman; *J.S. Flaherty* (US), Bozeman; *C.H. Draper* (US), NW of Redlodge, Carbon Co; *G. Davidse and A.W. Collotzi* 644 (GH, UC, US), 10 mi E Bozeman; *W.N. Suksdorf* 256 (WS), Jackrabbit Gulch, 10 mi Wilsall; (WS), Bozeman; *Nawrocki and Neff* 8 (UC), Missoula River; *W.E. Booth* 56269 (DAO), 12 mi S Choteau, Teton Co; 56202 (DAO), 15 mi S Conrad, Pondera Co; *A. Naasz* 1055 (DAO), Watkins, Prairie Co; *J.M. Gilbert and R.M. Taylor* 10927 (DAO), Lower St Mary Lake, 7 mi N of St Mary, Glacier Co; *E.W. Scheuber* (US), Yellowstone Park; (UC), Livingston; *R.S. Williams* 116 (US), Columbia Falls; *P.A. Rydberg and E.A. Bessey* 4541 (US), Briger Mtns, 7000'.

**North Dakota:** *J. Murdock* 3507 (GH), Spearfish Red Beds at mouth of Beaver Cr, 3700'; *W.P. Taylor* 351 (WS), Washburn; *J. Lunneell* (US, UC, GH, DS, RM), Butte, Benson Co; (PH, MO), Minot; (PH), Leeds; *R.P. Williams* 866 (MO), Emmons Co, 4 mi W of Temil; *W.C. Whitman* (US), Stark Co, Dickson; *J.F. Bergman* 1495 (GH), Church's Ferry; *O.A. Stevens* 131 (DAO, UC, RM), Berlin; 2388 (US), Kenmare, Ward Co; 2579 (UC, US), Dunseith, Rolette Co; *Fieldstad* (RM), Valley City.

**South Dakota:** *W.H. Forwood* 39 (US), Black Hills, Ft. Meade; *W.P. Carr* 9 (CAN, GH, MO, US), Newell; *S.S. Visher* 559 (RM), Date, Perkins Co; *A.C. McIntosh* 62 (RM), Tilford-Elk Creek, Piedmont; *P.L. Ginter* 36 (RM), Harney Forest, head of Wolf Canon, 6250';



*E.T. Wherry* (PH), Custer Co, 3 mi S of Custer St. Park; *C.A. Barr* 1063 (PH), Custer Co., 11 mi NW of Custer in Black Hills; 1064 (PH), 11 mi NW of Custer; 1061 (PH), 3 mi W Smithwick; 1060 (PH), 3 mi W Smithwick; 1062 (PH), cultivated, originally from S Custer Co 18 mi N Hot Springs; *J.F. Brenckle* (CAS), Northville; *E. Bondy* 376 (CAS), Ellis Co., near Cean Hill N of Hays.

**Colorado:** *H.D. Ripley* 5475 (CAS), 25 mi N Loma, W Garfield Creek, 6000'; *G.R. Hall* 348 (RM), White River Forest; *R.W. Pohl* 1949 (PH), Grand Co, S of Sulphur Springs; *C.R. Towne* T-85 (RM), Oray Co, Uncompahgre, 8500'; *G. Van Buren* GVB-22 (RM), Routte Co, Yampa, White R Forest, 7800'; *E.H. Graham* 9113 (DS), Moffati, near Price Creek, 6400'; 9129 (DS), Moffat, 10 mi W Youghall, 7700'; *G.E. Osterhaut* 2617 (RM), between Mecker and Craig; 2555a (RM), Minturn, Eagle Co; 2555 (RM), Minturn, Eagle Co; *C.F. Baker* 67 (UC, MO, WS), Gunnison Water Shed, Camarron, 6900'; *I. Tidestrom* 3420 (US), Mt Caribou; *C.S. Crandell* 469 (US), Howes Gulch; *L. Crawford* (GH, US), Routt Co, Steamboat Springs.

**Wyoming:** *A. Nelson* 7038 (RM, GH), Sand Creek, Albany Co; 4375 (GH, MO), Plambago Canon; 8235 (GH, MO, UC, US, DS, RM), Tie City; 8943 (GH, UC, US, PH, CAS), Laramie Hills; 19 (CAN, GH, MO, UC, US), Head of Pole Creek; 1215 (US, RM), head of Pole Creek; 43 (GH, US), head of Pole Creek; (RM), Moorcroft; 9612 (RM), Birds Eye; 4525 (US), Evanston; *K. Roach* (CAN), Tie City; *C.L. Porter* 3050 (GH, UC, US, DS, RM), Fox Creek, Albany Co; 8567 (DS, RM, GH, DAO, UC), Natrono Co, Casper Mtn, 8000'; 4886 (RM, DAO), N Platt R valley near Big Creek; *F.X. Jozwik* 64 (GH, RM), Casper Mtn, Garden Creek Falls, 6400'; *N.H. and P.K. Holmgren* 5017 (US), Lincoln Co, Twin Creek valley, old US hwy 30 N, 6750'; *L.M. Shultz* 2537 (MO), Lincoln Co, Salt Creel Pass, Bridger Nat Forest; *M. Ownbey* 553 (US, WS, MO, RM, DS), 7 mi NW Hulett, 4500'; *R.C. Rollins* 568 (WS), Johnson Co, Upper Johnson Creek, 4000'; *S. Stephens* 39523 (UC), Weston Co, 10.5 mi NE Four Corners; *R. Adams* (US), Upper Falls of the Yellowstone; *E.A. Mearns* 989 (US), Mommoth Hot Springs; *F. Tweedy* 408 (US), Mommoth Hot Springs; *F.H. Burgleshaus* (US), Mommoth Hot Springs; *M.S. Baker* (ALTA), Yellowstone Nat Park; *B. Maguire* 13596 (GH), Yellowstone Park, 10 mi N Yellowstone Falls; *D. Mason* (US), Ft. McKinney; *E. Stevenson* 73 (US), Uinta Co, La Barge; *I.J. Worthley* 137 (US), Big Horn Co.; *O'Murie* 257 (US), Jackson; *F.V. Hayden* (MO), Wind Rain valley, 5000-8000'; *C.W.T. Penland*



1827 (CAS), Summitt Co, 8500'; *C.R. Clark* 10 (RM), Albany Co, 6900'; *J.F. Macvride* 2720a (RM), Rock River, 7000'; *G.I. Sellon* 72 (RM), hills E of Laramie; *B.E. and L. Nelson* 398 (RM), Woods landing, S of Sheep Mt, 7800'; 1554 (RM), 20.5 mi WNW Lusk, 12.5 mi WNW Manville, 5200'; *R. O'Brien* 1029 (RM), 21 mi SW Wheatland; *J. Wetherell* 19 (DS), Pole Mt region, Happy Jack Rec Area, 8300'; 13 (DS, RM), Laramie Co, Laramie range, Happy Jack Road, 7500'; *W.J. Cockran C-150* (RM), Bighorn Co, Shell Ranger Station, 7750'; *R.L. Hartman* 2946 (RM), Carbon Co, 2 mi NNW Elk Mtn to Hanna, 7400'; *R.B. Current* 288 (RM), Shirley Basin 35 mi SSE Casper, 7110'; 309 (RM), Pathfinder Mtn, 34 mi SSE Casper, 7110'; *R. Schreibeis and K. Roman* 667 (RM), Fremont Co, Gas Hills NE Jeffery City; *O'lson, Gerhart, Rizer, Jones* 17 (RM), above Goose Lake, 11.5 mi NE Dubois, 7280'; *H.G. Fisser* 368 (RM), SW Thermopolis, Wind R Canyon, 5800'; *R.M. Hurd* 329 (RM), Bighorn For, road to Cull Watt Park, 7200'; *O.C. Harrison* 123 (RM), Star valley, Greys R., Afton area, 6000'; *R.E. Pfadt* 156 (RM), Niobrara Co, Manville; *E&D Pearson* 57 (RM), on Little Rocky, Clark's Fork valley, 5000'; *A Aldrich* 26 (RM), Sweetwater Co, NE Cedar Mt, 8120'; *V Willits* 12(406) (RM), Little Goose Valley; 406 (RM), Hanna Creek; 529 (RM), Hanna Creek; *R Lichvar* 92 (RM), Teton Co, Gros Ventre River; *RH Ohl* 24 (RM), Teton For, Buffalo Ranger Station, 6500'; *PW Danwiddie* 14 (RM), E Fork River near Boulder, 7000'; *JA Moore, LM Mayer, GG Reardon* 506> (RM), Uinta Co, 6880-7140'; *BL Coulter* 67 (RM), Teton Forest, Horsetail Ranger Station, 7300'; *LA Well* 66 (RM), between Fish and middle Piney Creek, 7500'; *EJ Williams F-17* (RM), Medicine Bow For, E Beaver Cr, 9000'; *FE McGrew* 175 (RM), Shoshone For, 6000'.

**Utah:** *BF Harrison* 7237 (MO), Toole Co, 10 mi S of Lolgreen; 8712 (DS), Wasatch Co, near East Portal, 8000'; 8313 (DS), Deer Cr Canyon, Utah Co, 7000'; *ME Jones* (DS, CAS, PH, UC, US, MO), *Echo*; *HM Christensen C-28* (RM), Sevier Co, Fish Lake For, Mud Flat, 7500'; *WB Miller* 2-24 (RM), Fish Lake For, Birch Cr Canyon, 7500'; *EH Graham* 8815 (DS, GH, US), Uintah Co, above Dry Fork, 6700'; 8084 (DS, MO), Diamond Mt plateau, 6500'; 8016 (DS, GH, UC), Duschne Co, Uinta R, 7200'; 8096 (DS, MO), Daggett Co, Grouse (summitt) Cr Canyon, 7000'; 9257 (MO), Wasatch Co, Horse Cr SW of Strawberry Reservoir, 7700'; *G Davidse* 343 (DAO, GH, UC, CAS, DS), Cache Co, 11 mi up Hyrum Canyon, 5400'; 1018 (PH), Wellsville Canyon, 5000'; 356 (GH, UC, US), 1 mi up left Fork Blacksmith Fork Canyon, 5700'; 364 (UC, US), .5 mi W Herd Hollow, 6000'; 375 (UC),



20.5 mi up Logan Canyon near Twin Cr Rd, 6200'; 417 (UC), 3 mi N of Hardware Ranch, 6000'; 1045 (US), Rich Co, 1 mi W Garden City, 6100'; 993 (UC), Box Elder Co, .5 mi E Deweyville, 4600'; 1011 (UC, US), Raft R Mtns, Sawtooth Natl For, 6200'; 316 (UC, US), Juab Co, Mt Nebo, 2 mi SE Mona; 330 (GH, UC), Utah Co, 3 mi SE Thistle, Manti-LaSal Nat For, 5400'; WC Muenscher, B Maguire 2384 (RM, GH, UC), 1 mi W Smithfield Sugar Factory; 2383 (UC), Spring Hollow, Logan Canyon; B Maguire 3576 (UC, RM, DS, PH), 3 mi E Logan; 3566 (MO, UC, RM), Boy Scout Camp, Logan Canyon, 5400'; CP Smith 2370 (DS, RM), Lewiston, Webster Spur; E Tucker 1171 (DS), Boy Scout Camp, Logan Canyon; 1136 (DS), hwy at Preston Camp, Logan Canyon; R Foster 7817 (RM), Duschne Co, Uinta River, 7200'; L Williams 559 (CAS, GH, MO), Daggett Co, Summitt Springs Ranger Station, 9000'; RT Best 18 (DS), Elko Co, Boy Scout Camp, 7200'; AR Standing 112 (RM), Uinta For, Lower Pole Canyon, 6700'; AE Aldous 3847 (RM), Manti Nat For, 6700'; C DeMoisy 9 (RM), Ashley Forest, Whiterocks, 6000'; M Burke 3567 (UC), Cache Co, Green Canyon, 5400'; AH Holmgren, RJ Shaw 7642 (US), San Pete Co, Manti Nat For; WC Clos 11 (US), Wasatch Mtns, Ephraim Canon, 2100m; I Tidstrom 1019 (US), Wasatch Mts, Ephraim Canyon, 7500'.

**Nevada:** HL Mason 4713 (UC), Cave Cr Post Office, Ruby Valley; 4760 (DS, GH, UC), 1 mi SW Cave Cr Post Office; AH Holmgren 812 (UC), Elko Co, Mt S of Railroad Canyon, 986 (UC), 18 mi NE San Jacinto; G Davidse and JL Gentry 1503 (GH, UC, WS, DS, RM, PH, DAO, MO), Pequop Mts, 5 mi W of hwy 40 and 30, 6790'; 1099 (UC, US), Humbolt Co, NE Winnemucca, 6100'; 1639 (GH, UC, US), Humbolt Nat For, 6100'; H Engelmann 122 (MO), W slope Seftor valley, 6600'; AE Hitchcock 891 (US), Kingston canyon and Birch Cr, 2250-2800m; FS Goodner and WH Henning 131 (UC), Lander Co, Birch cr S of Austin; P Train 2757 (UC), Kingston Co, E Toiyabe Range, 5500'; GE Moore M-311 (RM), White Pine Co, Nevada For, 8000'; M-610 (RM), New For, Rock Sprint near Hamilton, 8000'; C McMillan and KH Knight 59 (RM), 40 mi E Ely; MA King (CAS), Warm Springs, White Pine Co; JH and YM Robertson 49 (CAS, RM), Elko Co, Humbolt Nat For, Clover Cr, 5900'; SL Glownke 11023 (PH), Lincoln Co, Spring valley 5 mi W Ursine; JM & MAR Linsdale 38M (CAS), Kingston Cr, Landon Co, 7500'; 893 (CAS), Nye Co, S Twin River, 7300'; HD Ripley & RC Barneby 6020 (CAS), N Wachre Co, 7 mi SW Vyr, 6000'.

**Arizona:** HH Rusby 523 (UC, MO), Ash Fork, AT.



**Kansas:** *E Bondy* 515 (MO, US), Hays.

**Washington:** *ZP Tammer* 8 (WS), Lincoln Co, Creston Site Inventory; *TH Scheffer* (WS), Grant Co, E side upper Grand Coulee; *RG Jeffery* (WS), Adams Co, 2 mi N of Macall; *GR Vasey* (US), Washington Territory; *V Batie* (WS, RM), Okanogan Co, Twisp; *V Duthie* (DS), Spokane Co, near Spangle; *Sandberg & Leiberg* 47 (DS, CAS, US, MO, UC, GH), Hangman Cr, Spokane Co; *HT Rogers* 350 (CAS, DS, PH, ALTA, US, UC, MO, WS, GH), Ferry Co, Columbia River, 10 mi N Hunters Ferry, 1290'; *CS Williamson* (PH), Almota; *CV Piper* 1715 (RM, GH, US, WS), Pullman; *A Radigan* 91 (RM), Colville For, 3300'; *ME Jones* (DS), Davenport; *WN Suksdorf* 8530 (MO, WS, UC), SE Spangle; 1895 (GH, WS), Spokane Co, Spangle; 9584 (WS), Klickitat Co, Binden; 10200 (DAO), E of Husum; *J Gleason* 245 (WS), W of 7 Mile and N Reardon; *M Whicker* (WS), Williams Lake; *Hull & LF Anderson* 2499 (GH), Campus, Pullman; *WR Hull* 418 (WS), Pullman; *FL Moore* (MO), Pullman; *GH Jones* 1500 (WS), Whitman Co, .5 mi N Pullman; *A Eastwood & H St John* 13220 (WS), Whitman Co, Winona; *H St John, Cary, FL Pickett, FA Warren* 6262 (WS), Rock Cr, W Winona; 6274 (WS), Rock Cr, W of Winona; 6887 (WS), Rock Lake; 6931 (WS), Palouse R, 2 mi E Hooper; *FG Meyer* 174 (MO), near Wawawai; 1436 (GH, MO, US), S end Rock Lake; *K Whited* 1216 (US, WS), Douglas Co, Waterville; (WS), Ellensburg; 279 (US), Kittitas Co, 6 mi S Ellensburg; *CB Fiker* 588 (WS), Okanagan Co, Lime Belt; 649 (WS), Blue Lake, in Lime Belt, W of Omak; *OT Edwards* 213 (WS), Patterson Lake, W of Winthrop; *FL Pickett* 473 (WS), Grant Co, Coulee City; *E Zaring* 79 (WS), Grant Co, Mtn E of Steamboat Rock, Grand Coulee; *UH Zuberbahler* (WS), Buttercaves, 2 mi W Trout Lake; *GH Ward* 293 (US), Chelan Co, St Louis Ranch, N of Chelan; *JA Calder, RC Taylor & JA Parmelee* 16116 (DAO), Lincoln Co, 6 mi SE Creston, hwy 2; *R Daubensmire* 59174 (WS), Lincoln Co, 9 mi N Davenport.

**Oregon:** *WC Cusick* (WS), E Oregon; 1608 (DS, GH, UC, US), E Oregon; 1653 (DS, GH, MO, UC, US), prairies of S Blue Mts; 3156 (GH, MO, US), eastern Oregon; 1849 (DAO, GH, UC, US, WS), eastern Oregon; 3760 (WS), Union Co, Hog Mt, 4000'; *AW Sampson* 233 (RM), Wallowa Nat For; *DC Ingram* 1589 (RM), Ololla Ranger Station, Cascade For, 5000'; *T Howell* 353 (PH, CAN, GH, US), Harney valley; *LF Henderson* 9244 (CAS), Harney Co, Burns; 9245 (DS, CAS), Malhuer Co, N Malhuer River, Scotts; 5194 (CAS, DS, MO), Grant Co, Prairie City; *EH Reid and LF Henderson* (RM), Grant Co, PNW Whitman For, 6000';



*NP Gale* 142 (DS), Gilliam Co, near Condon; *JB Leiberg* 2172 (GH, UC, US), Owyhee, Malheur Divide, 1250m; *RD Cooper* (WS), Riley; *ME Peck* 20707 (UC), 18 mi W Burns, Harney Co; 25209 (UC), 3 mi S Frenchglen; 26133 (DAO), N of Burns; *JH Bartholf* (MO), Camp Harney; *OV Deming* 27 (GH), Guard Cr at the Post, Hart Mtn Nat Antelope Refuge.

**Idaho:** *G Davidse* 1036 (UC, US), Bear Lake Co, 1.3 mi W Ovid; 1757 (MO), Butte Co, W Atomic City; *JF MacBride & EB Payson* 3055 (GH, RM), Martin, Blaine Co; *A Cronquist* 2432 (GH, MO), Blaine Co, 9 mi N Ketchum; 2275 (GH, MO), Bannock Co, 3 mi S Pocatello, 4500'; *CL Hitchcock & CV Muhlick* 8810 (CAN, GH, UC, WS, MO, DS, RM), Custer Co, 20 mi N Sun Valley; *CL Hitchcock* 23633 (WS, UC, DS, RM), between Challis and Mackay; *A Nelson & JF Macbride* 1812 (GH), Owyhee Co, House Creek; *EB & LB Payson* 1803 (GH, MO, CAS, RM), Lemhi Co, Salmon; *DA Saunders* 4425 (US), Lincoln Co, Shoshone; *A Nelson* 10057 (GH, MO, UC, RM), Victor; *RL Lingenfelter* 605 (DAO, GH, WS, UC), Mink Cr Canyon, 5 mi S Pocatello; 532 (WS), Clark Co, 1947 burn area, US sheep expl station; *RJ Davis* 1895 (UC, WS), Pocatello; *E Palmer* 40 (US), Pocatello; *B Maguire* 2386 (GH), Franklin Co, 6 mi N Preston; *W Trelease* 4424 (US), Shoshone Falls; *CD Marsh* (US), Soda Springs; *JH & CD Christ* 19618 (WS), Cassia Co, 13 mi SW Oakley; *CC Parry* 34 (GH), Snake Co; *EH Quayle* 13 (DS, RM, UC), Juniper Hills, 10 mi W St Anthony; *JF Macbride* 11 (RM, GH, MO), Montpelier; *FA Barkley & R Blondeau* 4004 (UC, PH), Clark Co, S of Humphrey; *Olson & Bergstien* (DS), Bannock Co, City Creek; *ME Soth* P-8 (RM), Pocatello; *R Foster* 6081 (RM), Caddy canyon; *J Pechaea & GD Pickford* 33-8 (RM), Dubois, 5600'; *HH Van Winkle* VW-5 (RM), Lemhi For, Warm Springs Ranger Station; *GA Miller* M-63 (RM), Lemhi For; *AH Wheeler* 4 (RM), Salmon For, Boyle Cr Basin; *FW Godden* 13 (RM), Salmon For; *DE Romano* 13 (RM), Salmon For; *CF Cusick* 13 (RM), Lemhi For, Mohogany Cr R Stat, 7600'; *OW Mink* 66 (RM), Lemhi For, Warm Spring R Stat, 6800'; *JG Koock* JK-54 (RM), Targhee For, Table Rock, 5800'; *M Anderson* 4 (RM), Caribou For, Maple Hollow, 4900'; *JO Stewart* c-23 (RM), Cache For, Maple Hollow; *S Welsh* 16655 (RM), Oneida Co, 8 mi W Holbrook; *D Bennett* 9 (RM), Twin Falls; *JF Pechance* 35-37 (RM), Fremont Co, burning project; *H Work* 528 (RM), Custer Co, Challis For; 562 (RM), Custer Co, Challis For, Morgan Cr, 5800'; *CB Kock* 37 (RM), Challis For, Big Hill near divide, 9000'; *NH & PK Holmgren* 4781 (CAS), Bear R Co, 2.2 mi W Bloomington, 6100'; *EE Stock* 162 (RM), Minidoka For, Noonie Place, 6000'.



*Viola vallicola* subspecies *vallicola*

**CANADA, Alberta:** *D Fabijan* 572 (ALTA), Chin Lake 23 km S Taber, 3000'; 524 (ALTA), Nose Hill, Calgary, 3700'; 550 (ALTA), Little Fish Lake, 3600'; 567 (ALTA), Spruce Coulee, Cypress Hills, 3600'; 516 (ALTA), Leitch Collieries, Crowsnest Pass, 4500'; 517 (ALTA), 1 km S of Bow Crow Forest Entrance, S of Hillcrest, 5500'; 519 (ALTA), Lynx Creel, 19 km S of Hillcrest, 4500'; 520 (ALTA), Castle River, S of Hillcrest, 4500'; 523 (ALTA), Beauvais Lake, 4500'; 533 (ALTA), Nose Hill, Calgary, 3700'; 551 (ALTA), Hand Hills, N of Little Fish Lake, 3000'; 556 (ALTA), 3 km E Cereal on hwy 9, 2600'; 545 (ALTA), .5 km W Turner Valley, 4000'; 548 (ALTA), 5.7 km E of Cluny on hwy 1, 2800'; 568 (ALTA), Whitla Coulee, .3 km E of Etzikom turnoff on hwy 3, 2700'; 565 (ALTA), 4.6 km N of Golden Prairie turnoff on hwy 41, 2700'; 566 (ALTA), Medicine Hat, 2300'; 547 (ALTA), Bow Crow For entrance 10 km W Turner Valley on hwy 546, 4800'; 276 (ALTA), Waterton, 4400'; 268 (ALTA), 8 km W Longview on hwy 541, 4300'; 194 (ALTA), Etzikom Coulee, 38.6 km S Taber on hwy 36, 3100'; 256 (ALTA), Porcupine Hills, 8.7 km E of hwy 22 towards Nanton, 4500'.

**Saskatchewan:** *D Fabijan* 579 (ALTA), 1.8 km SW of Cypress Hills Park, 4100'; 590 (ALTA), 3.7 km S of Moose Jaw.

*Viola vallicola* subspecies *major*

**CANADA, British Columbia:** *D Fabijan* 489 (ALTA), 3.6 km E Cawston, 3000'; 487 (ALTA), Courtney Lake, 3500'; 488 (ALTA), Princeton, 2500'; 486 (ALTA), 11.4 km S of Kamloops toward Lac La Jeune; 499 (ALTA), 13.3 km E of Grand Forks, 2000'; 507 (ALTA), Johnston Creek Park, 3000';

**UNITED STATES, Oregon:** *D Fabijan* 471 (ALTA), 57.7 km S of Washington State border on hwy 3.



*Viola praemorsa* subspecies *praemorsa*

**CANADA, British Columbia:** JR Anderson 198.5 (DAO), Thetis Lake, Vancouver Island; AJ Pineo (UC), Victoria; J Hett & W Armstrong 12 (UBC), above Royal Oak peat bog, VI; JK Henry (UBC), Victoria; 9110 (CAS), Victoria; AJ Hill 204 (DAO), Victoria; GM Dawson 33940, (CAN), Vancouver Island; J Macoun 2421 (CAN, GH), vicinity of Victoria; 2467 (CAN), Cedar Hill, near Victoria; 87046 (CAN), Beacon Hill, near Victoria; 78,459 (US), vicinity of Victoria, VI; Dr Lyall (GH), near Nanimo, VI; JA Calder & A Szczawinski 28523 (DAO), small park in Victoria; PP Henson (DAO), Uplands, Victoria; MC Melburn (DAO), westside Uplands Park, Victoria; CF Newcombe (GH), Observatory Hill, VI.

**UNITED STATES, Washington:** D Fabijan 414 (ALTA), Tacoma; 394 (ALTA), Lyle; JW Thompson 14278 (GH), Klickitat Co, near Centerville; 11373 (GH), near Lyle; 5137 (GH, MO, US), Pierce Co, near Tacoma; 8238 (CAN, DS), near Roy; W Suksdorf 22 (CAN, WS), Mt Bingen, Bingen; 5552 (WS), Bingen; 5563 (DAO, WS), near Bingen; 9565 (WS), Klickitat Mt near Rockland; 9579 (WS), Bingen; 100 (GH), Klickitat Co, woods; 10828 (WS), Skamania Co, Hood; 10829 (WS), Skamania Co, Hood; UH Zuberbuhler (UC), Buttercaves, 2 mi W Trout Lake; WJ Eyerdam (MO, UC), Pierce Co, Niqually Prairie; JM Grant (UC), Lakeview; L Benson 1204 (DS, MO), Pierce Co, south Tacoma, 300'; Brandell (MO), prairies, Tacoma; AJ Leroas (DS, UC), South Tacoma, W end 66 St; JB Flett (UC), Tacoma; MS Baker 9634 (DAO, MO, WS), South Tacoma, near old Municipal Auto Court; OD Allen 101 (GH), Roy; RM Horner 58 (WS), Waitsburg; R59B83 (GH, US), Waitsburg; NL Gardner 30 (WS, UC), Whidbey Is.

**Oregon:** LF Henderson 86 (DS, UC), College Campus, Forest Grove; 5872 (DS, CAS, MO), near Selma; JW Thompson 2140 (DS), Jackson Co, E of Brownsboro; 527 (DS), Washington Co, Forest Grove; 567 (MO), summitt David's Hill, Forest Grove; 2030 (DS), Roseburg; 4124 (DS, GH, MO, US), Salem; MS Baker (DS), Kirby; 5442 (DS, UC, US), Josephine Co, Grant's Pass; 5441 (UC), near Medford; E/ Applegate 4061 (DS), Ashland Co, Keene Creek Ridge Ashland-Klamath Falls hwy; MW Gorman 4416 (DS, WS), Salem; JC



*Nelson* 23 (DS), Polk Co.    *L Dale* (DS), Gilbert Creek near Grants Pass; *L Constance* (CAS, UC), Lane Co.    Coburg, N of Eugene; (UC), vicinity of Eugene; (UC), Spencer Butte, S of Eugene; *EW Hammond* 27 (GH, US), Jackson Co, near Wimer; *JO Steward* 16 (US), Horsefly valley, Klamath Co, 4800'; *WJ Spillman* 21 (WS), Monmouth; *Watkins & Dunn* 448 (MO), Umatilla Co, S hwy 395 Camas Cr and N Fork John Day River; *W Cusick* 3956 (WS), Douglas Co, Looking Glass; *Drake & Dickson* (MO), Scappoose.

**California:** *WA Weber* 12294 (DAO), Humbolt Co, Van Duzen River, W of Dinsmore, 2400'; *MS Baker* 8059 (GH, MO, UC, CAS, DS), Humbolt Co, headwaters of Yager Cr, 2 mi W Bridgeville on Kneeland hwy; *JP Tracey* 4159 (UC), near summit Buck Mt, 6067 (UC), head of south Yager Creek; 6609 (UC), Lawrence Creek, Kneeland pasture, 2500'; 8730 (DS, JEPS, UC), Kneeland pasture, 2500'; 12566 (UC), Hoopa Mt, near summit, W of Hoopa, 3500'; 18607 (UC), Van Duzen R, opposite Buck Mt, 2500'; *AM Alexander & M Kellogg* 4662 (DS, UC), Modoc Co, Warner Mts S of Eagleville, 7000'; *MPE Ames* (US, GH), Plumas Co; *A Eastwood* 899 (MO), Shasta Co, Goose valley; *R VanDeventer* 335 (JEPS), Del Norte Co, Beer's Rd at Flume crossing; *LE Smith* 88 (US), Siskiyou Co, Sisson; *GD Butler* 1325 (DS, UC, US), Siskiyou Co, Bulls meadows, Goosenest Mt; *S & CH Quibell* 2852 (UC), Fresno Co, Ball Diamond meadow, off 168 between Shaver L and Rock Haven; *HP Chandler* 1288 (CAS, DS, UC, US), Hupa Indian Res, 3600'; *JG Lemmon* 48 (GH, UC), Sierra valley; *DK Kildale* 5185 (DS), Humbolt Co, Chalk Mt; 5975 (DS), Horse Mt, A *Eastwood & T Howell* 5410 (CAS), Madera Co, Bass Lake; *E Belteff* (CAS), Burney, Shasta Co.

#### *Viola praemorsa* subspecies *linguaefolia*

**CANADA, Alberta;** *D Fabijan* 648 (ALTA), Carthew Summit, Waterton, 7500'; 654 (ALTA), Boivin Lake, 6800'; *AJ Breitung* 16670 (DAO, UC), Waterton, Carthew Pass, 8000'; 13935 (ALTA), Mt Carthew; 16999 (DAO), Crypt Lake, S facing slope; 17275 (ALTA), Avion Ridge, S facing cirque; *FW Hunnewell* 15560 (GH), Waterton, Crypt Lake, 6800'; *EH Moss* 3335 (ALTA), Waterton, Cameron Lake; *JG Packer* 3716 (ALTA), Waterton, bank of Bertha Creek, by bridge; 4175 (ALTA), above Goat Lake, 6500'; *J*



*Kuijt, J Nagy, M Gadd* 3662 (CAN), Carthew summit, scree slope; *4043* (CAN), W of Lost Lake, E facing scree slope, 7000'.

**UNITED STATES, Washington:** *D Fabijan* 398 (ALTA), 5.8 km E Husum.

**Oregon:** *E Applegate* 46 (US), Swan Lake Valley; 2281 (US), Jackson Co, head of Kear Creek; *JP Rose* 1692 (MO), Pine ridge, Klamath Co; *LF Henderson* 640, 1924 (MO), Hood R Co, open pine woods; *CL Hitchcock* 20531 (CAN, DAO, WS), Wheeler Co, 15 mi N Spray on hwy 207; 20524 (CAN), 5 mi SW Lonerock; *GW & GD Douglas* 3366 (DAO), 12 mi W Mitchell, 4500'; *A Cronquist* 6340 (WS), 15 mi NE Spray; *G Davidse & AW Collotzi* 506 (GH), Harney Co, 8 mi SE Frenchglen to Fish Lake, 6000'; *WC Cusick* 1987 (GH, US, WS), Harney Co, higher Steins Mtns; 3165 (MO, WS, US), Union Co, Bank of East Eagle Cr, Wallowa Mt, 1330m; 1876 (GH), eastern Oregon; *FH Whittaker* SS237 (WS), Josephine Co, Lake Mt, Grayback area, Siskiyou Mts, 6500'; *ME Peck* 25985 (WS), Baker Co, 4 mi SE Halfway.

**Idaho:** *D Fabijan* 622 (ALTA), Red Rock Pass, 7000'; *EB & LB Payson* 1973 (GH), Summit to base of Mt NE Henry Lake; *JF Macbride* 952 (GH, US, WS), Silver City, Owyhee Co, 7000'; *CL Hitchcock, RV Rethke, R VanRaadshooven* 3852 (WS), Yellowstone NP, 1 mi below Flat Rock Camp, S of Wentrona, 6500'; *NH Holmgren* 5578 (CAS), Bonneville Co, Big Elk Mt, S of Palisade Dam, 8800'.

**Montana:** *D Fabijan* 631 (ALTA), McDonald Pass, 6325'; *DT MacDougal* 819 (US), MacDougal Peak, 6800'; *O Thompson* (US), Glacier Park Station; *A Cronquist* 7934 (GH), Missoula Co, Holland Lake, 7500'; *CL Hitchcock* 16378 (GH, UC, WS, DS), Park Co, Silver Pass, 10 mi W Four Mile Ranger Stat; 16651 (DS, GH, DAO, WS, US), Park Co, near Lookout Stat, 3 mi W Veartooth Lake; 16510 (CAN, WS), Sweengrass Co, 2 mi below Rainbow lake; 16828 (CAN, UC, WS), Madison Co, S side Black Butte, 10,000'; 16919 (CAN, GH, US, WS), Red Hill, Gravelly Range, 9500'; *JG Witt* 1188 (WS), Cooke Co, 3 mi E Cooke City, 8000'; 1665 (DAO, GH, WS), 2 mi E Cooke City, 8000'; 1705 (DAO, GH, WS), 4 mi N Cooke City; *DW Swingle* (DAO), Gallatin Co, Bozeman, Monument Mt, 9500'; *EJ Moore* (GH, WS, US), Bridger Mts; *PA Rydberg & EA Bessey* 4539 (US), Bridger Mts; 4540 (GH), Madison Co, Old Hollowtop, Pony Mts, 8500'; *CL Hitchcock & CB Muhlick* 12755 (DS, CAS, WS), Beaverhead Co, W Oreamnos Lake, Anaconda Range; 12953 (CAS, GH, MO, UC), Pioneer Range, between Sheep and Black Lim Mts, 9000'; 12114 (WS), Meagher



Co, Checkerboard Cr, Castle Mts; 12343 (DS, CAS, MO, WS), .5 mi S Yogo Peak, Little Belt Mts; *LM Umbach* 81 (DS, GH, US), Coulees, Midvale; 106 (DS, US), Tavines, Midvale *JF Morton & JM Venn* NA4839 (CAN), Cookes City at edge of Yellowstone; *W Sundell* 2 (CAS), Missoula Co.

**Colorado:** *CF Baker* 225 (GH, MO, UC, WS), Grand Mesa, Gunnison Watershed, 9000'; *GE Osterhout* 2694 (GH), Mts E of Steamboat Springs; *LN Gooding* 1572 (DS, GF, MO, UC, US), Rabbit Ear Range; *SA Sponberg* 62-34 (US), Gunnison Co, N of Mtn Biol Lab, Gothic; *WW Egg/estan* 90 (GH, MO, US), Kebler Pass, 3050m; *J Langenheim* 591-48 (UC), Trail to Virginia Basin, 105000'; *F Rama/ey* 10537 (UC), East Fores Lake near Tolland; *Lethel, Wil/ey & Clokey* 4205 (CAN, MO, UC, US, WS), Boulder Co, Lake Wildora, 2850m; *GN Jones* 34155 (UC), Yankee Doodle Lake, 10700'; *TS Bandegee* 13234 (MO), Mt Carbon, Elk Mts; *E Payson* 370 (GH), Tabeguache Basin, 8000'; *I W Clokey* 4209 (UC), Hefferson Co, Clear Cr, 1578m; *I Tidstrom* 3420 (UC), Mt Caribou; *NH Russell* 59-72 (DS), Gunnison Co, Gothic; 59-74 (CAS), Emerald Lake, Gothic.

**Wyoming:** *EA Mearns* 638 (US), Melvin Gulch, Mammoth Hot Springs; *AE Porsi/d* 16956 (CAN), Park Co, Mt Washburn, Yellowstone NP, 10000'; *G Davidse & AW Collotzi* 676 (GH, UC, US), 32 mi S Red Lodge on hwy 212; 670 (UC), Lincoln Co, summit hwy 89 S of Afton; *B Venrick* 173 (MO), Dunraven Pass, Yellowstone NP, 8600'; *RG Sto/ze* 871 (GH), Beartooth Mts, Clay Butte, 2800m; *EB Payson & GM Armstrong* 3352 (GH), 5 mi E Afton; 3647 (GH), Redmount, NE Smoot, 9600'; *RA & A Nelson* 84 (DS, MO, WS), U of W summer camp, Medicine Bow Mts, 10,000'; *A Nelson* 7914 (GH), Medicine Bow Mts; 110005 (WS), Medicine Bow Mts; *GT Goodman* 627 (MO), Medicine Bow Mts; *LC Anderson* 252 (UC), Teton Co, Treasure Mt scout camp, 11 mi E Driggs; *R Will/iams* 24 (MO, WS), STS Ranch, Moose, 6700'; 2174 (UC, WS), Double Diamoun Ranch, Moose, 7000'; *EB & LB Payson* 2713 (GH, UC), Sublette Co, Piney Mt, 25 mi W Big Piney; 2783 (GH, MO, UC, US), 15 mi E Merna; *JH Beaman & KJ Stone* 1402 (DAO), Carbon Co, near Bridger Peak, Sierra Madre Range, 10,700'; *M Ownbey* 844 (DS, WS), Big Horn Co, near Medicine Mt, 9500'; 107 (DS), Albany Co, U of W summer camp, Medicine Bow Mts, 9500'; *LO & R Williams* 3077 (GH, MO, WS), 10-15 mi E Kane, 8500'.

**Utah:** *EH Graham* 8147 (GH, MO, US, DS), Dushesne Co, E slope Wolf Creek Pass, Uinta Basin, 9000'; *ME Jones* 6175 (DS, MO, UC, US), Crystal Mine, Marysvale, 9000'; *E Tucker*



1203 (DS), Cache Co, Brush Canyon, Wellsville Mts; *S Clark & K Taylor* 2435 (DS), Emery Co, E of Cleveland Reservoir, 8500'; *CP Smith* 2370 (CAS), Cache Co, Lewiston-Webster spur; *G Davidse* 1726 (CAS), Bear R Mts, Twin Creek.

**Nevada:** *NH & PK Holmgren* 4898 (CAS), Humbolt Co, Independence Mts, 29 mi NNW Elko, 6400'; *A Nelson & JF Macbride* 1942 (GH, MO, US), Jarbridge, 8500'; 2040 (GH), Jarbridge, upper corral Creek, 7000'; *AA Heller* 10547 (GH, US), Ruby Mts, Star canyon near Deeth, 8500'; *M & GB Ownbey* 2808 (DAO, WS), Humbolt Co, Buckskin Mtn, 12 mi S & 14 mi E McDermitt; *JL Gentry & G Davidse* 1640 (DAO), Santa Rosa Range, 33.5 mi NE US 95 and Nev 83, 6960'; *Holmgren, JJ Fay & BL Benthers* 4327 (UBC), Black Rock Range, Mahogany Cr Canyon E of Summit Lake, 7200'.

**California:** *L Whitney* 1739 (UC), Modoc Co, Happy Camp, 15 mi W Canby, 4800'; 1738 (UC), Crowder Flat, 5000'; *RM Austin* 453 (US), Goose Lake valley; *LE Smith* 690 (GH, US), Sisson; *A Eastwood* 361 (GH, US), Deer Park, Lake Tahoe region.

#### *Viola praemorsa* subspecies *f/avovirens*

**UNITED STATES, Washington:** *D Fabijan* 478 (ALTA), 1.5 km S and 4.5 km E Anatone; 375 (ALTA), Klickitat Valley, N of Maryhill on hwy 97; *WC McCalla* 5413 (ALTA), Kittitas Co, Lake Cle Elum.

**Oregon:** *Gilliet & Taylor* 11013 (CAN, DAO), Wallowa Co, 8 mi S of Washington border on hwy 3; *NP Gale* 52 (MO, GH), S of Myrtle Cr, Douglas Co.

**Idaho:** *E McKay* (WS), Vollmer; *M Ownbey* 2043 (DS, DAO, CAN, UC, US, ALTA), Idaho Co, 1 mi E Kooskia; *MS Baker* 7408 (CAS, DS, DAO, MO, WS), Clearwater River, Kamiah, 1500'; *HA & EG Heller* 3156 (CAS, DAO, DS, MO, RM, UC, WS), Lake Waha, Nez Perce Co, 2000-3500'; *Sandberg, MacDougall, Heller* 222 (DS, MO, UC, US), Lake Waha; *Sharsmith* 3534 (UC, WS), Lake Waha; *M Ownbey* 2043 (MO), 1 mi E Kooskia, Idaho Co; *JF McBride* 860 (DS), Boise Co, Squaw Cr, 3500'; *W Burns* 52 (WS), Latah Co, Potlatch Canyon Kendrick; *JH Christ* 18268 (WS), Idaho Co, Lower slopes Mt Idaho; *J Gleason* 449 (WS), Idaho Co, near Grangeville; *CL Hitchcock & CV Muhlick* 8422 (DS, GH), Clearwater Co, 2 mi N of Cavendish; *LF Henderson* (DS, US), bluff above Juliaetta.



**Montana:** *W Sunde* // 2 (DAO, GH), Missoula Co; *Burdick* 5 (DAO), Missoula Co, 10 mi S Missoula on Bitterroot R.

**Wyoming:** *EB Payson & GM Armstrong* 3288 (MO), E of Afton, 7500'; *L Williams* 1091 (MO), Double Diamond Ranch, Teton Co, 7000'; 663 (UC), Grand Teton NP, Jenny Lake Canyon, 7500'.

*Viola bakeri*

**UNITED STATES, Washington:** *D Fabijan* 396 (ALTA), Trout Lake, 12,325'; *WC McCalla* 5358 (ALTA), Klickitat Co, N of Husum, on White Salmon River; 6222 (ALTA), Calaveras Co, Big Meadow, valley floor, 6800'; 6324 (ALTA), near Summit Lake, Lassen NP, 6700'; *W Suksdorf* 1894 (WS), Klickitat Co, Falcon valley.

**Oregon:** *E Applegate* 10532 (DS), Union Rock, Crater Lake NP; 8317 (DS), Klamath Co, Grizzly Hill, Swan Lake Valley; 6164 (DS), Josephine Co, Grayback Mt; 10424 (DS), Josephine Co, Lake Mtn Trail; 5764 (DS), Warner Valley, E Mt Lassen; *GE Kelly* (CAS), Crater Lake; *VL Crosby* 589 (DS), Josephine Co, 15 mi E Cave Junction; *EP Sheldon* 12580 (DS), Lane Co, Lake Valley; *CL Hitchcock and JS Martin* 5245 (DS), Josephine Co, Lake Mt Trail, 4 mi E Oregon Caves, 6400'; 4863 (DS), Deschutes Co, 4 mi N North Sister Mts, MacKenzie Pass, 6800'; 4855 (DS), Deschutes Co, 6 mi W MacKensie Pass, 4800'; *JW Thompson* 13040 (CAS), Jackson Co, Huckleberry Mt, Rogue Range Nat For, 3000'; *A Cronquist* 7851 (CAS), 9 mi N Sisters, Trout Cr, 4500'; *RS Ferry & R Duthie* 462 (DS), Crook Co, Tumalo Ranger Station, 6700'; *JT Howell* 7156 (CAS), Deschutes Co, Sparks Lakes.

**California:** *MS Baker* 5215 (CAS, DS, UC, US), Placer Co, hwy 40, 1.5 mi W Big Bend Ranger Station; 8406 (DS, UC), Nevada Co, 5 mi W Bowman Lake, 6000'; 8403 (DS), Placer Co, Squaw Cr near hwy to Taho; 8388 (DS), Sierra Co, Webber Lake; 8377 (DS), Tehama Co, hwy 89, 6000'; 8052 (DS, CAS), Mt Lassen hwy (89) near checking station, 5800'; 8556 (UC), Amador Co, S end Silver Lake, 7290'; 10232 (CAS), Glenn Co, Black Butte, 6700'; *ER Drew* (DS), Sierra Co, Independence Lake; *EB Babcock & GL Stebbins* 1576 (DS), Yuba Pass, 1818m; *BJ Jorgensen* 477 (DS), Little Truckee Rd between



Independence and Webber Lakes; *HK Wagnon* 1684 (DS), Webber Lake; *A Head* (DS), Gold Lake, 6700'; (CAS) Plumas Co, Gold Lake, Feather R region, 6300'; (CAS) Plumas Co, Lake Center camp, Feather R region, 6300'; *CC Bruce* 1276 (DS), Shasta Co, Mt Lassen; *JT Howell* 36558 (CAS), Dusch Meadows, 6500'; 36574 (CAS), Tehama Co, Sulphur works station, Lassen P, 6600'; 27610 (CAS), Jamison Cr, 6200-6400'; 35675 (CAS), Drakesbad, 5300-6000'; 35900 (CAS), Drakesbad, 5300-6000'; *H Leschke* (CAS), Cold Boiling Lake, Lassen NP, 7550'; *A Eastwood* 870 (CAS), Shasta Co, Mt Lassen; 7001 (CAS), Portola; *BF Jackson* (CAS), Yuba Pass; *Kellogg* (DS, G), Placer Co, Cisco; *ME Jones* (DS), Emigrant Gap; (DS), Plumas Co, 13 mi W Prattville, 4500'; *AA Heller* 9859 (DS), Placer Co, Summit, 7000'; *BJ Jorgenson* 515 (CAS, DS), Nevada Co, Soda Springs; *MS Baker & HK Wagnon* 13012 (DS), Theama Co, 6.1 m3 NW Cohillack Camp; *CR Quick* 50-25 (CAS), Mill Cr Meadows W of Gurnsey Camp, Lassen N For; *HC Cantelow* (CAS), S of Bucks Lake; *RJ Weatherby* 1438 (CAS), Lake Almanor, 4650'; *R Baergabipi* 1733 (DS), Plumas Co, Little Grey Eagle Cr, Gold Lake region, 6700'; *FN Bennett* (CAS), Butte Co, Jonesville; *WL Atkinson* 1900 (DS), Edorado Co, Pyramid Peak; *GT Robbins* 2019 (CAS), Eldorado Co, 4 mi S of Wrights Lake, 6700'; *L Beane* 1842 (DS), Fresno Co, Huntington Lake, 7000'; *E Carter* 64 (DS), Fresno Co, Huntington Lake, 7000'; *MS Jussef* (G, CAS), Camp Baxter, 20 mi from Darringtons; *LS Rose* 40438 (CAS, UC), Nevada Co, Soda Springs, 6700'; 54053 (CAS), 3 mi W Soda Springs; *FA MacFadden* (CAS), Donner Lake; *DD Keck* 4363 (DS), Mariposa Co, Yosemite NP, 2375m; *A Eastwood & JT Howell* 4999 (CAS), Trinity Co, Scott Mts N of Carrville; *JP Tracey* 10596 (DS), Humbolt Co, Trinity summit, 6000'; *AM Vollmer & L Beane* 9 (DS), Siskiyou Co, Scott Mtn; *RS Ferris & L Lorraine* 11712b (DS), Siskiyou Co, Plowman's (Noyes) valley, E fork Scott R; *HP Chandler* 1688 (DS), Marble Mt, 8000'; *DE Breedlow* 3366 (DS, CAS), hwy 89, 8 mi E McCloud, 3700'; *L Rowntree* (CAS), 40 mi E of McCloud, 4000'; *A Eastwood* 1107 (CAS), McCloud; *ED Cantelow* 1454 (CAS), Scott Mtn, 5000'.













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